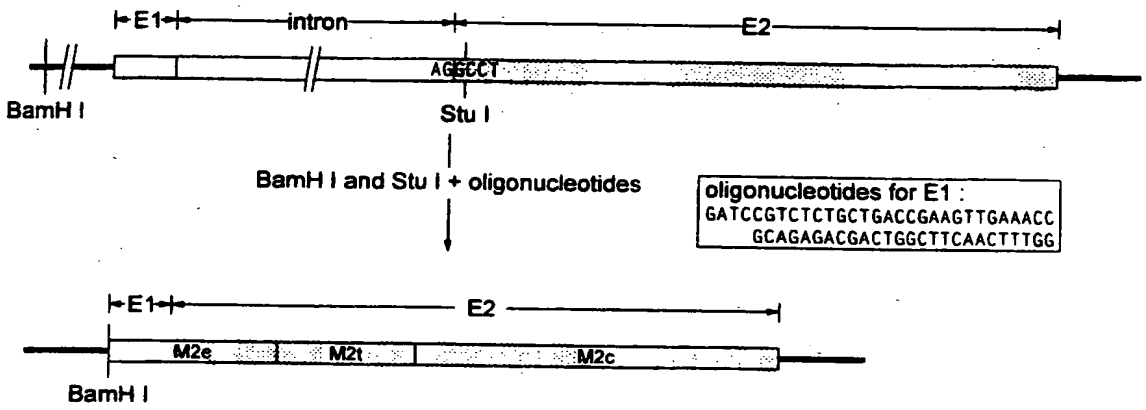
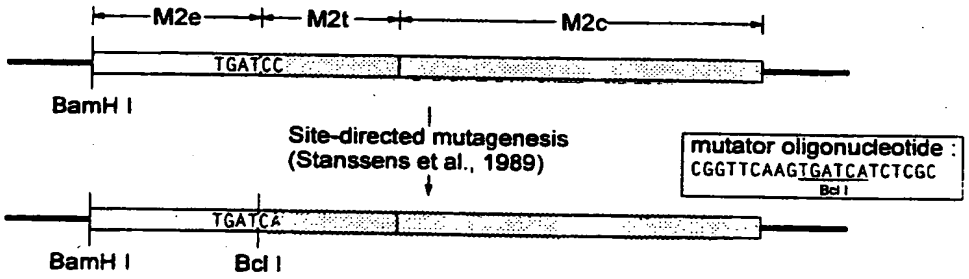
Figure 1

A



B



C

	2	3	4	5	6	7	8	9	10	11
Nucleotide sequence :	TCT	CTG	CTG	ACC	GAA	GTT	GAA	ACC	CCT	ATC
Amino acid sequence :	Ser	Leu	Leu	Thr	Glu	Val	Glu	Thr	Pro	Ile

12	13	14	15	16	17	18	19	20	21	22	23	24
AGA	AAC	GAA	TGG	GGG	TGC	AGA	TGC	AAC	GGT	TCA	AGT	GAT
Arg	Asn	Glu	Trp	Gly	Cys	Arg	Cys	Asn	Gly	Ser	Ser	Asp

figure2a1

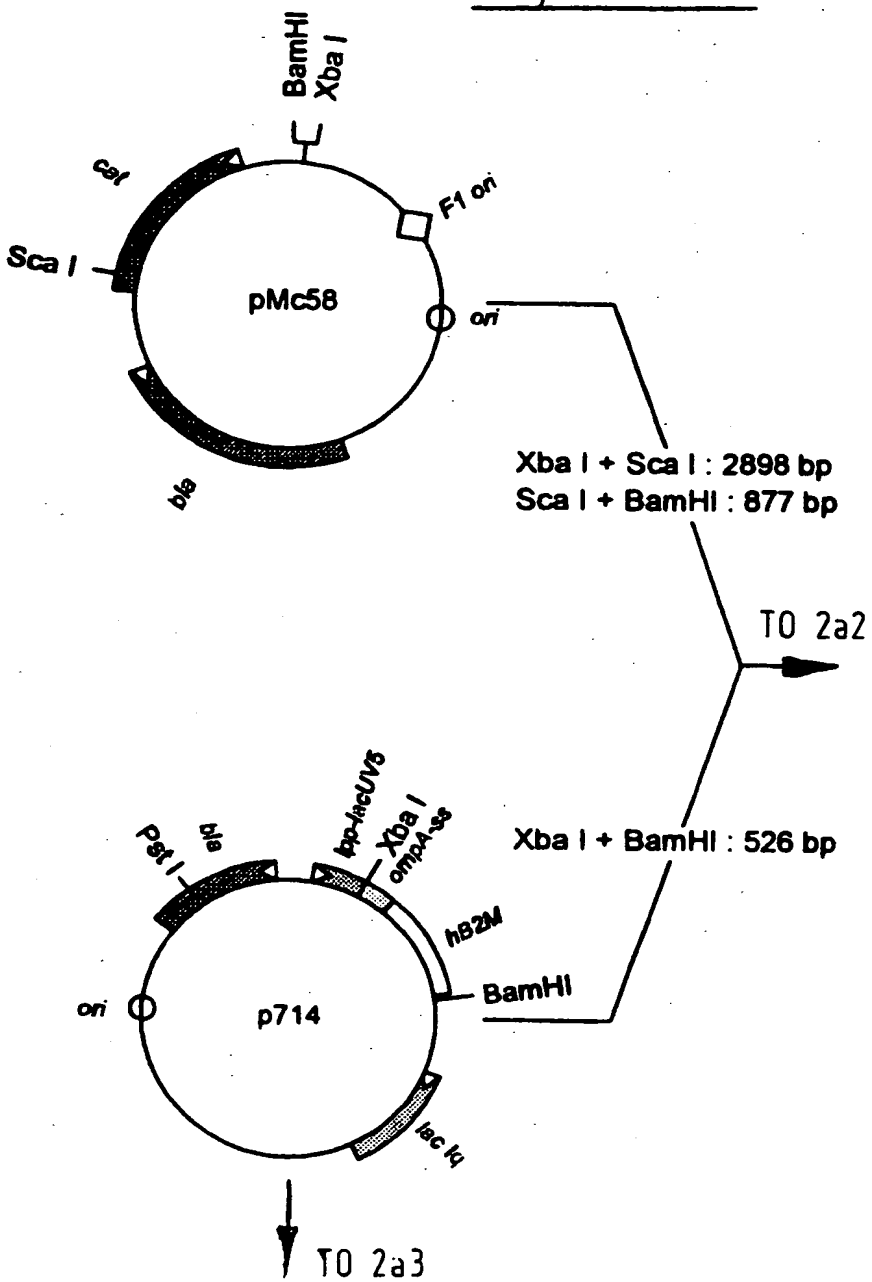

BamHI
Xba I
cat
F1 ori
Sca I
pMc58
ori
bla
Xba I + Sca I : 2898 bp
Sca I + BamHI : 877 bp
TO 2a2
Xba I + BamHI : 526 bp
Pst I
bla
bpt-lacUV5
Xba I
ompA-ss
hB2M
BamHI
ori
p714
lac Iq
TO 2a3

figure2a2

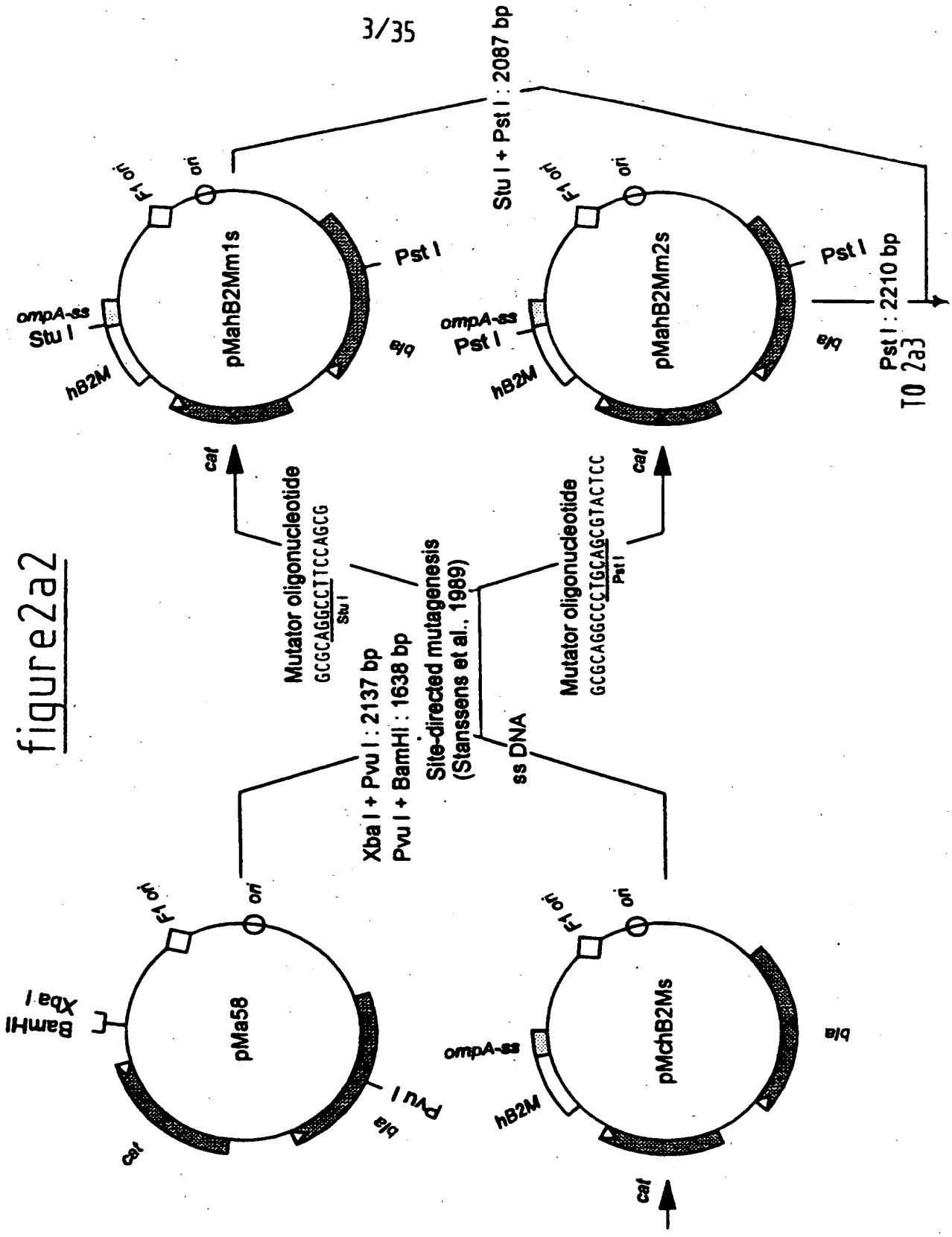

pMa58
BamHI
Xba I
Pvu I
bla
cat
F1 ori
ori
pMchB2Ms
ompA-ss
hB2M
bla
cat
Xba I + Pvu I : 2137 bp
Pvu I + BamHI : 1638 bp
Site-directed mutagenesis
(Stanssens et al., 1989)
ss DNA
Mutator oligonucleotide
GCGCAGGCCTTCCAGCG
Stu I
Mutator oligonucleotide
GCGCAGGCCCTGCAGCGTACTCC
Pst I
pMahB2Mm1s
ompA-ss
Stu I
hB2M
Pst I
bla
cat
pMahB2Mm2s
ompA-ss
Pst I
hB2M
Pst I
bla
cat
Stu I + Pst I : 2087 bp
Pst I : 2210 bp
TO 2a3

figure2a3

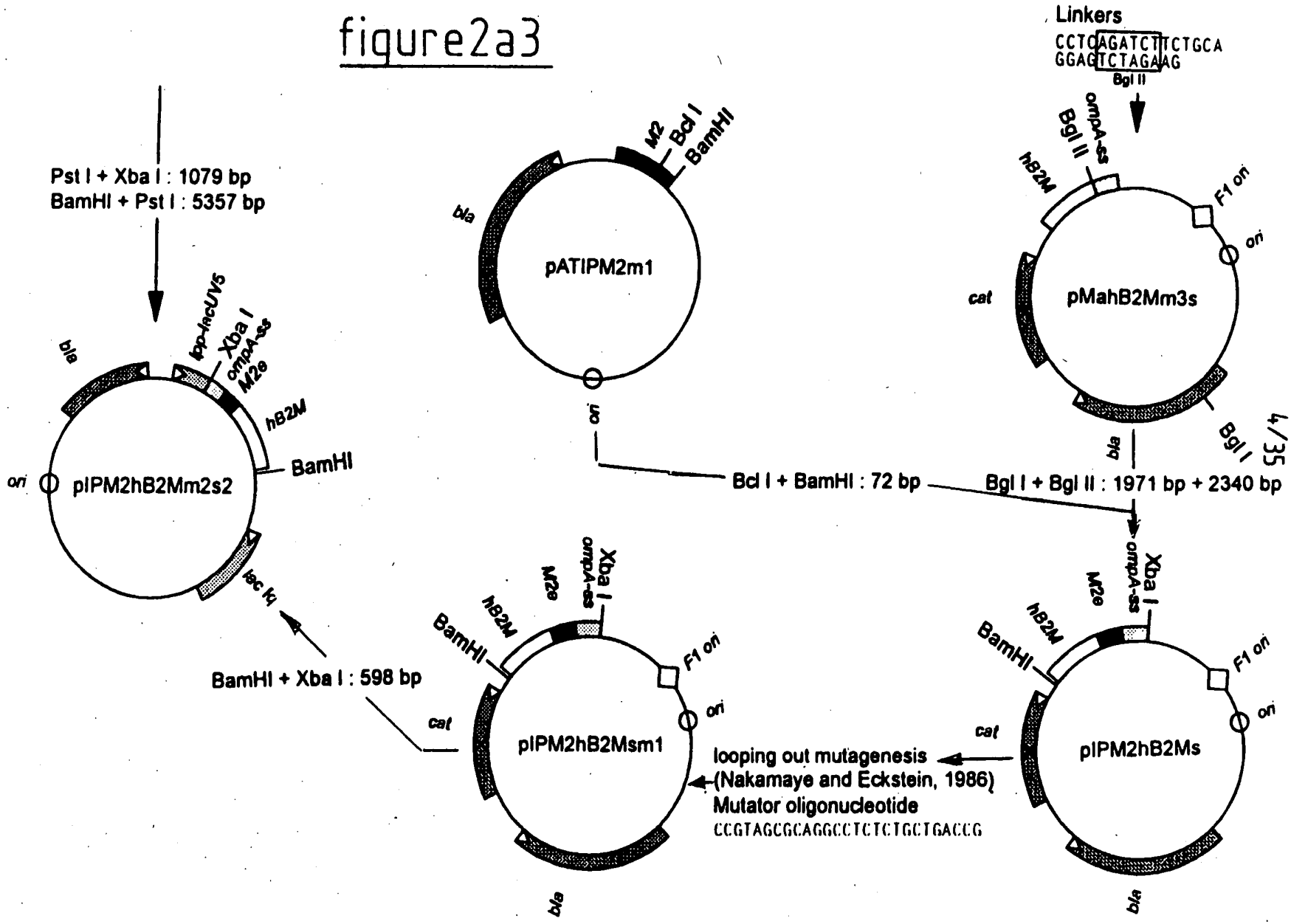

Linkers
CCTCAGATCTTCTGCA
GGAGTCTAGAAG
Bgl II
Pst I + Xba I : 1079 bp
BamHI + Pst I : 5357 bp
pATIPM2m1
M2
Bcl I
BamHI
bla
ori
pMahB2Mm3s
hB2M
Bgl II
ompA-ss
F1 ori
ori
cat
bla
Bgl I
pIPM2hB2Mm2s2
bla
tpp-lacUV5
Xba I
ompA-ss
M2e
hB2M
BamHI
ori
lac Iq
Bcl I + BamHI : 72 bp
Bgl I + Bgl II : 1971 bp + 2340 bp
BamHI + Xba I : 598 bp
pIPM2hB2Msm1
BamHI
hB2M
M2e
Xba I
ompA-ss
F1 ori
ori
cat
bla
pIPM2hB2Ms
BamHI
hB2M
M2e
Xba I
ompA-ss
F1 ori
ori
cat
bla
looping out mutagenesis
(Nakamaye and Eckstein, 1986)
Mutator oligonucleotide
CCGTAGCGCAGGCCTCTCTGCTGACCG

Figure 2b

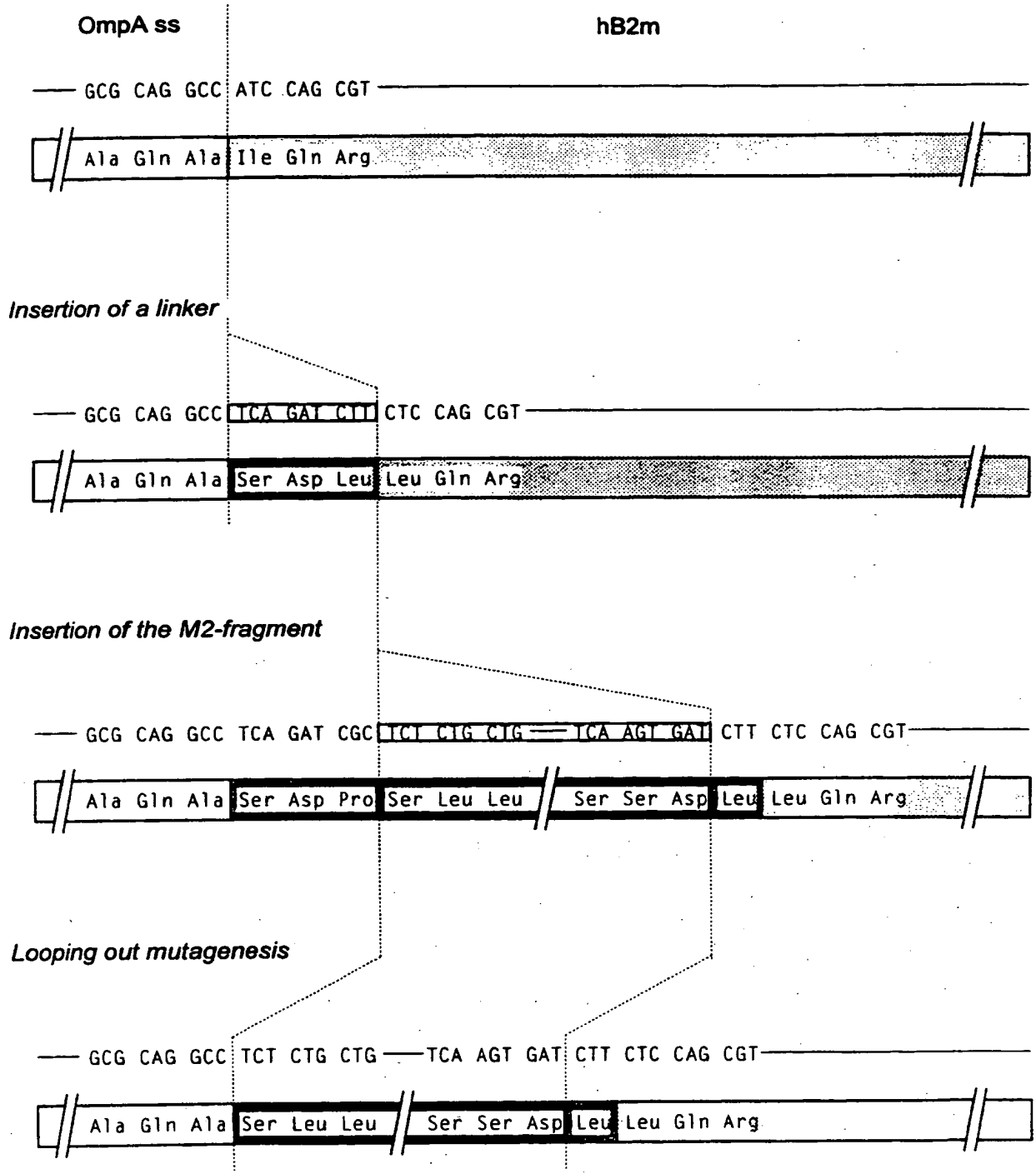

OmpA ss
hB2m
GCG CAG GCC ATC CAG CGT
Ala Gln Ala Ile Gln Arg
Insertion of a linker
GCG CAG GCC TCA GAT CTT CTC CAG CGT
Ala Gln Ala Ser Asp Leu Leu Gln Arg
Insertion of the M2-fragment
GCG CAG GCC TCA GAT CGC TCT CTG CTG TCA AGT GAT CTT CTC CAG CGT
Ala Gln Ala Ser Asp Pro Ser Leu Leu Ser Ser Asp Leu Leu Gln Arg
Looping out mutagenesis
GCG CAG GCC TCT CTG CTG TCA AGT GAT CTT CTC CAG CGT
Ala Gln Ala Ser Leu Leu Ser Ser Asp Leu Leu Gln Arg

figure 3a1

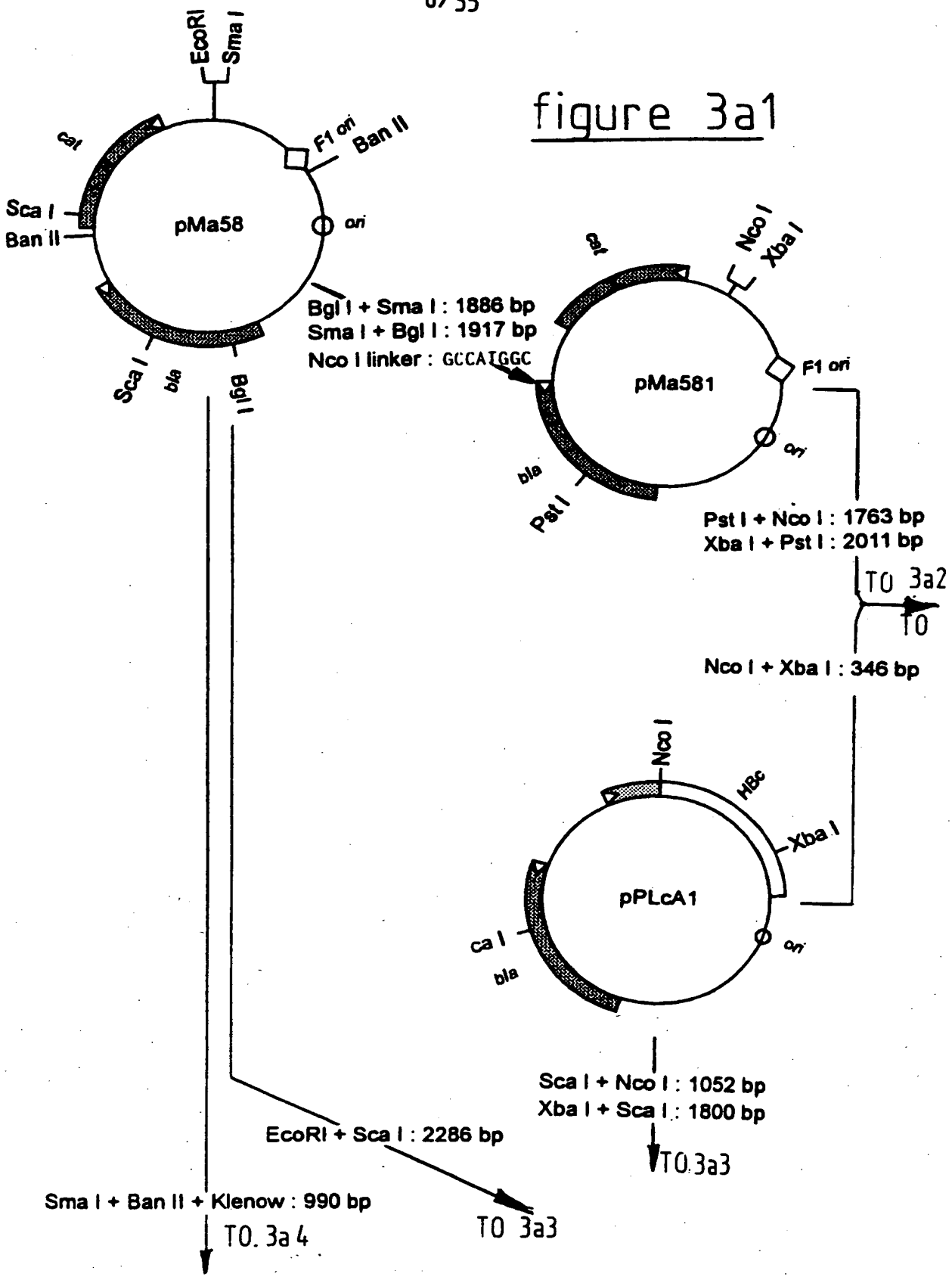

EcoRI
Sma I
cat
F1 ori
Ban II
Sca I
Ban II
pMa58
ori
Sca I
bla
Bgl I
Bgl I + Sma I : 1886 bp
Sma I + Bgl I : 1917 bp
Nco I linker : GCCATGGC
Nco I
Xba I
cat
pMa581
F1 ori
ori
bla
Pst I
Pst I + Nco I : 1763 bp
Xba I + Pst I : 2011 bp
TO 3a2
TO
Nco I + Xba I : 346 bp
Nco I
HBc
Xba I
pPLcA1
ca I
bla
ori
Sca I + Nco I : 1052 bp
Xba I + Sca I : 1800 bp
TO 3a3
EcoRI + Sca I : 2286 bp
TO 3a3
Sma I + Ban II + Klenow : 990 bp
TO. 3a 4

figure3a2

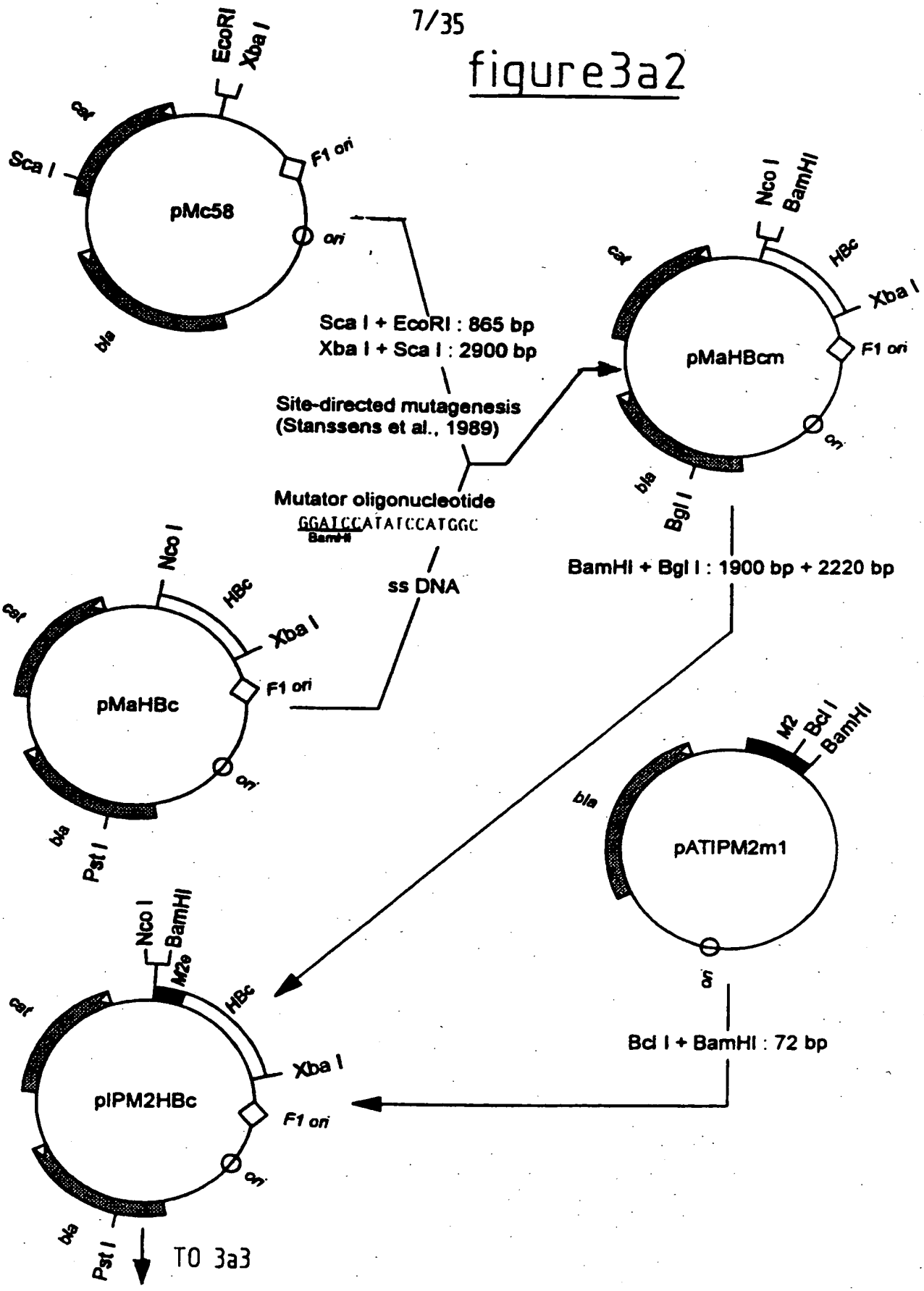

pMc58
EcoRI
Xba I
Sca I
cat
F1 ori
ori
bla
Sca I + EcoRI : 865 bp
Xba I + Sca I : 2900 bp
Site-directed mutagenesis
(Stanssens et al., 1989)
Mutator oligonucleotide
GGATCCATATCCATGGC
BamHI
ss DNA
pMaHBcm
Nco I
BamHI
HBc
Xba I
F1 ori
Bgl I
BamHI + Bgl I : 1900 bp + 2220 bp
pMaHBc
Nco I
HBc
Xba I
F1 ori
Pst I
pATIPM2m1
M2
Bcl I
BamHI
δ
Bcl I + BamHI : 72 bp
pIPM2HBc
Nco I
BamHI
M2e
HBc
Xba I
F1 ori
Pst I
TO 3a3

figure3a3

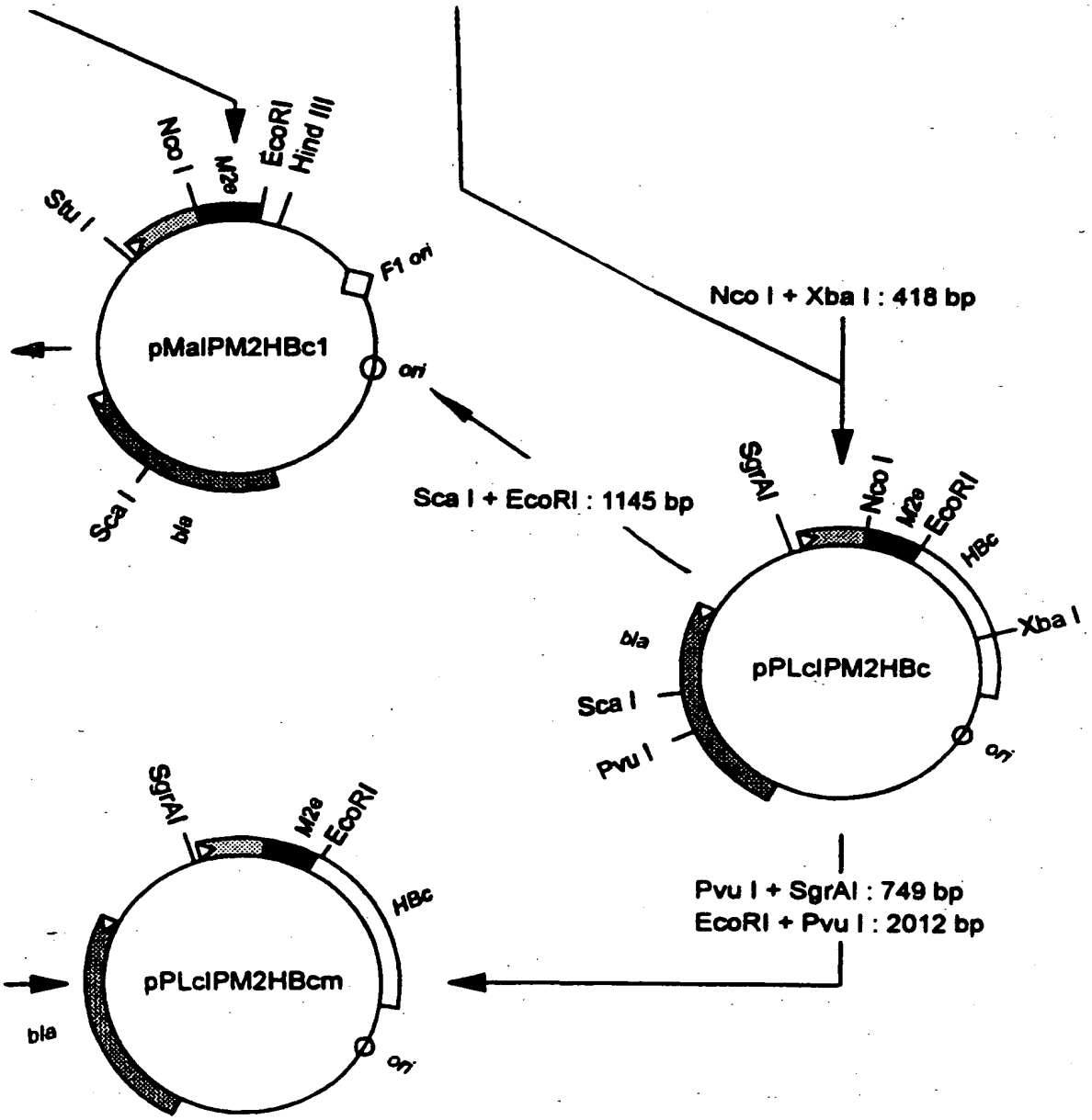
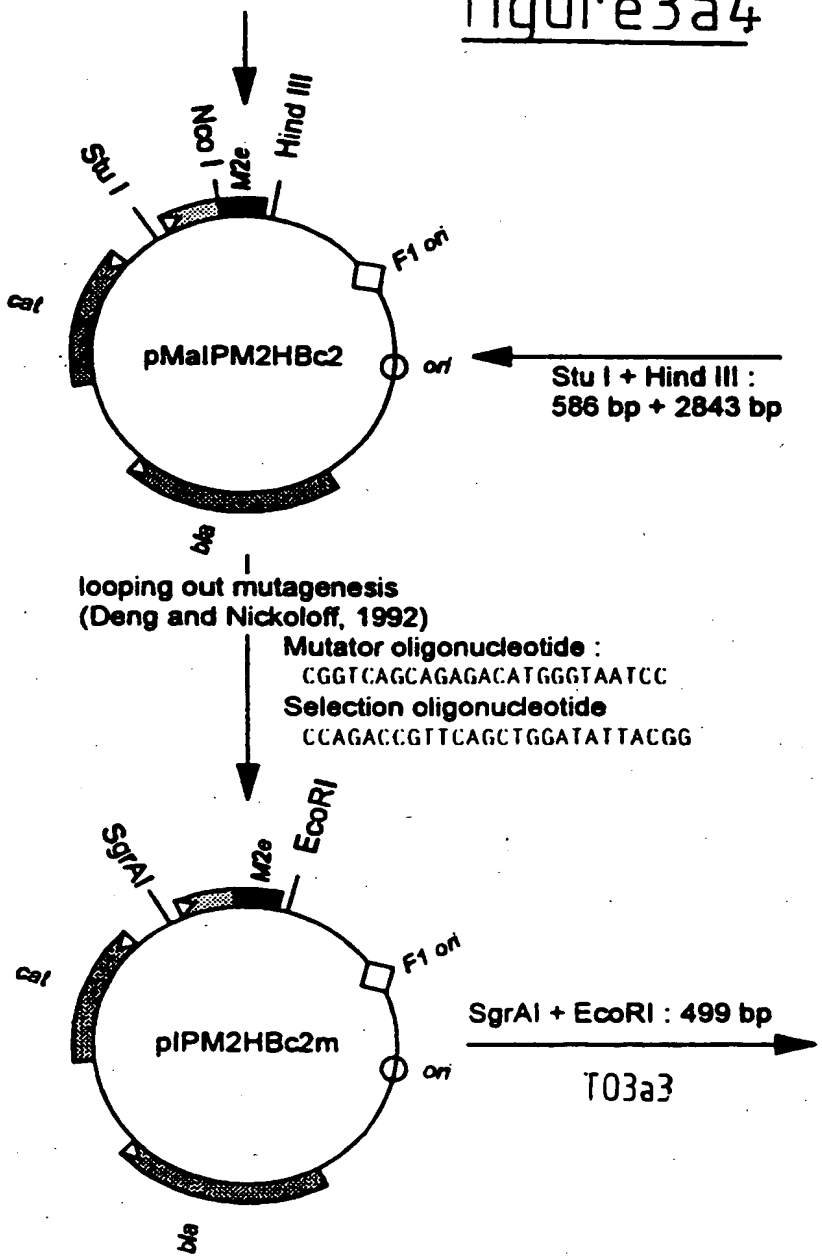

Nco I
M2e
EcoRI
Hind III
Stu I
F1 ori
pMalPM2HBc1
ori
Sca I
bla
Nco I + Xba I : 418 bp
Sca I + EcoRI : 1145 bp
SgrAI
Nco I
M2e
EcoRI
HBc
Xba I
bla
pPLclPM2HBc
Sca I
Pvu I
ori
SgrAI
M2e
EcoRI
HBc
pPLclPM2HBcm
ble
ori
Pvu I + SgrAI : 749 bp
EcoRI + Pvu I : 2012 bp

figure3a4



SUBSTITUTE SHEET (RULE 26)

Figure 3b

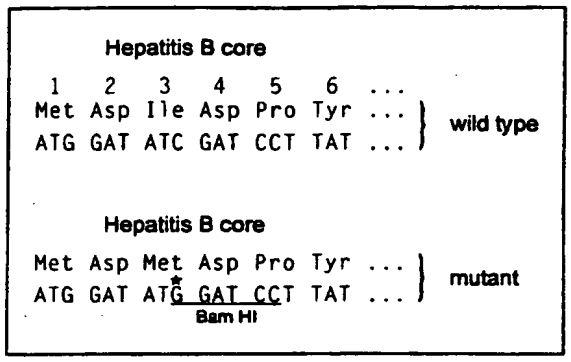


Figure 3c

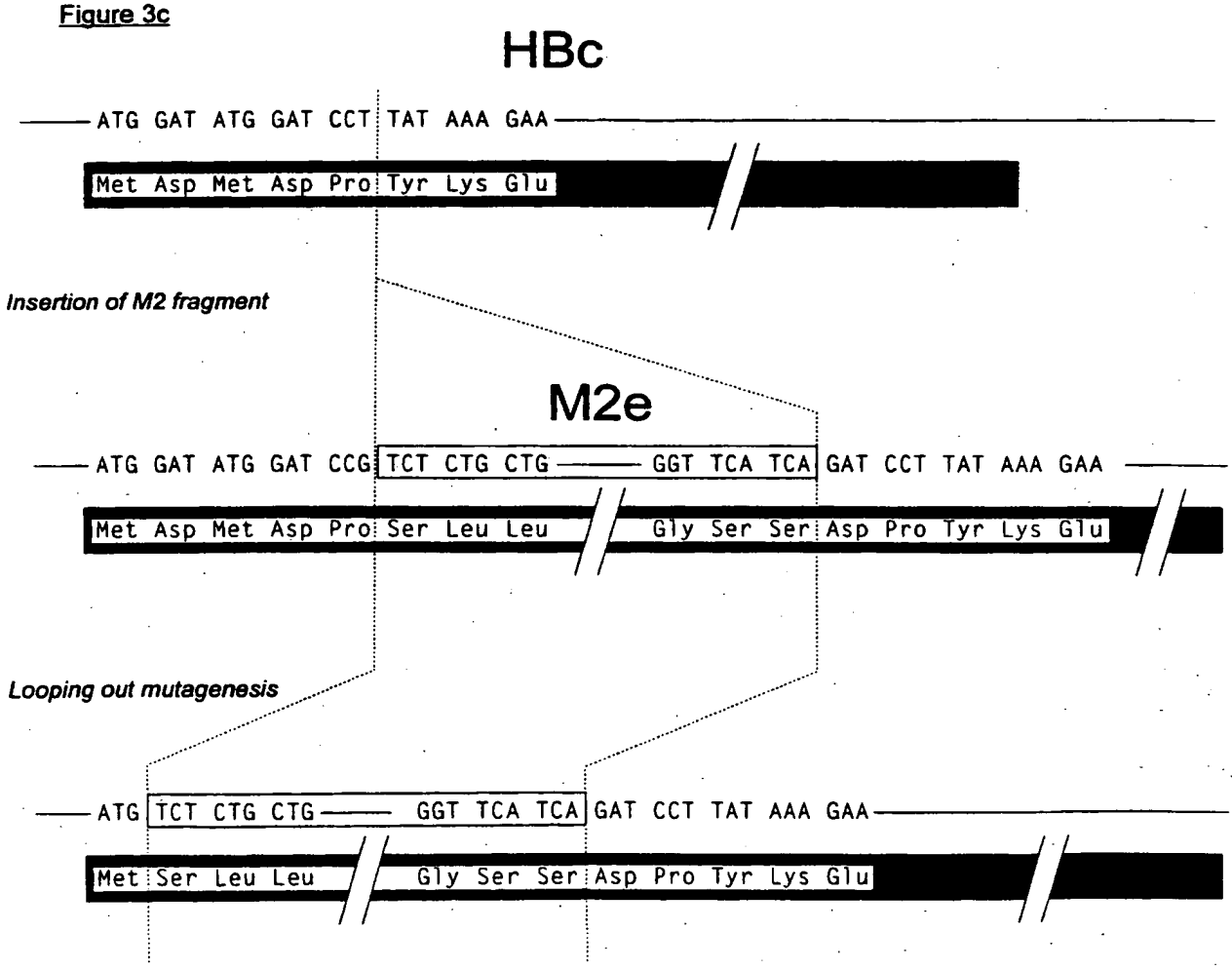


Figure 4

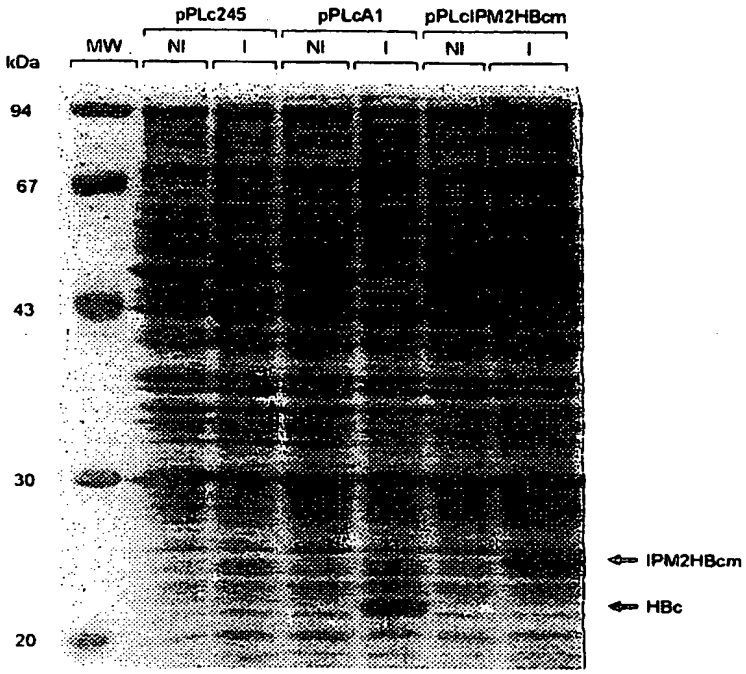
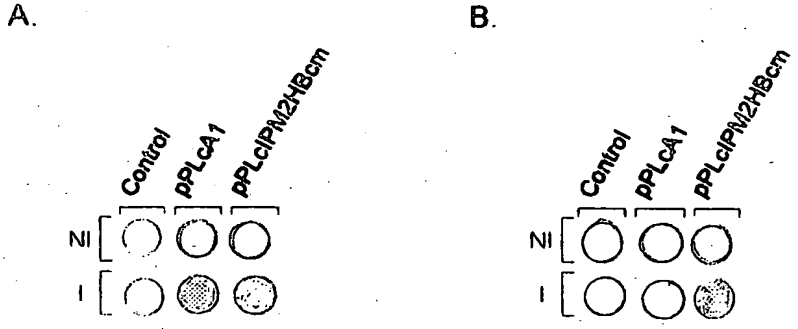


Figure 7



Corrected Sheet

Figure 5

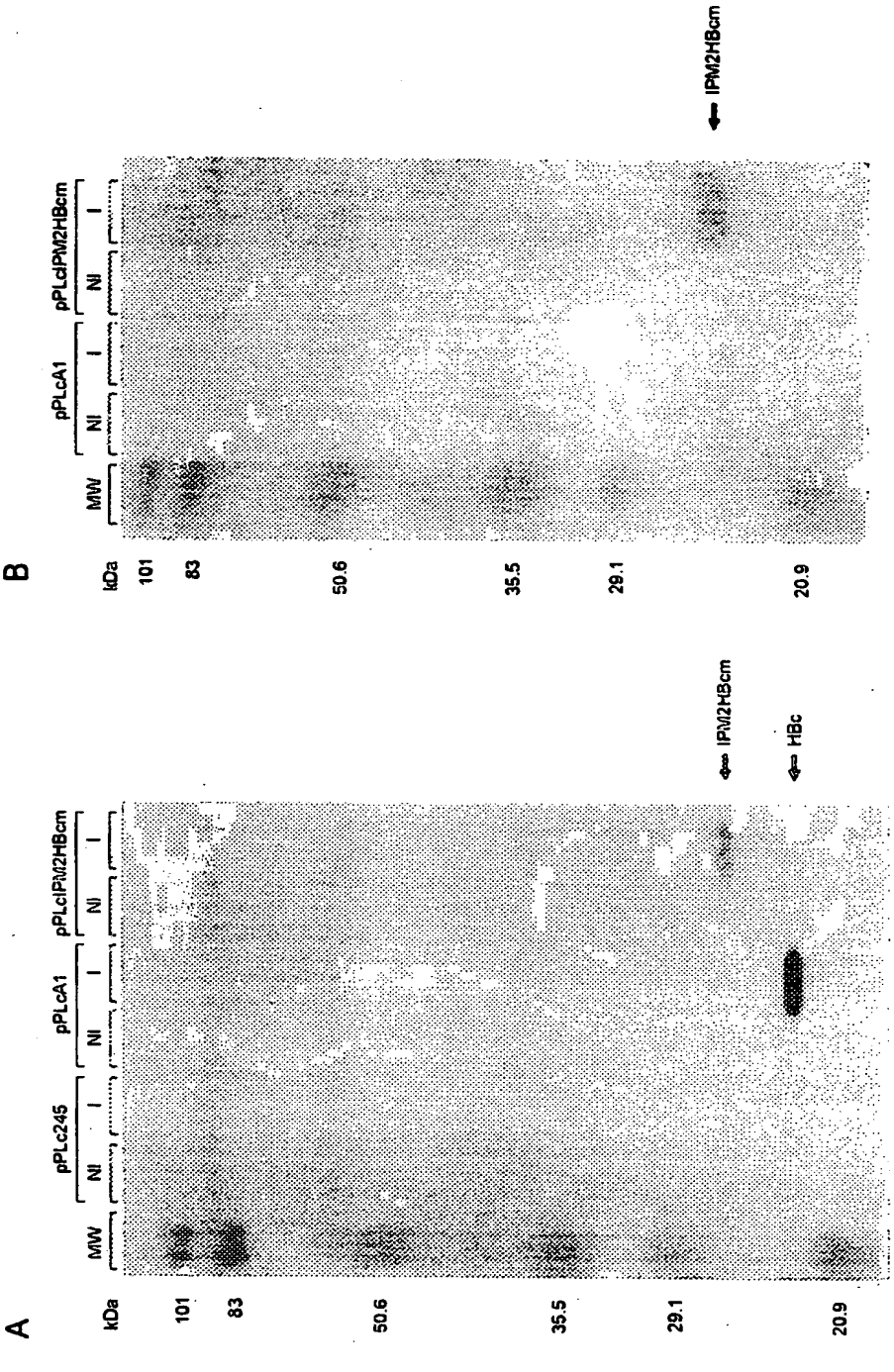


Figure 6

ATG TCT CTG CTG ACC GAA GTT GAA	Nucleotide sequence of *ipm2hbcm*
Met Ser Leu Leu Thr Glu Val Glu	Translated amino acid sequence
Ser Leu Leu Thr Glu Val Glu	Amino terminus of the fusion protein IPM2HBcm
Ser Leu Leu Thr Glu Val Glu	Amino terminus of the M2 protein of A/Udorn/72

SUBSTITUTE SHEET (RULE 26)

Figure 8

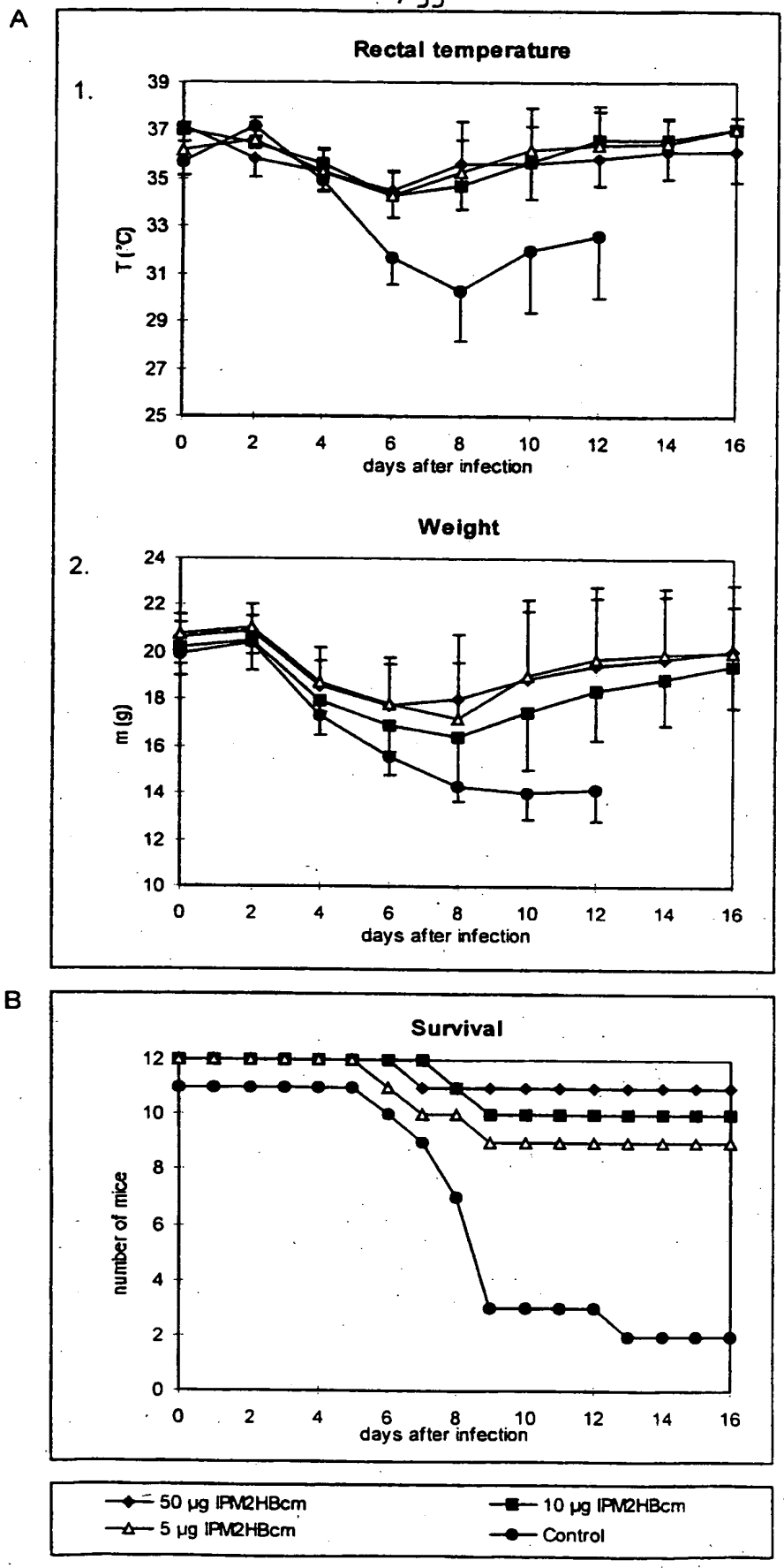
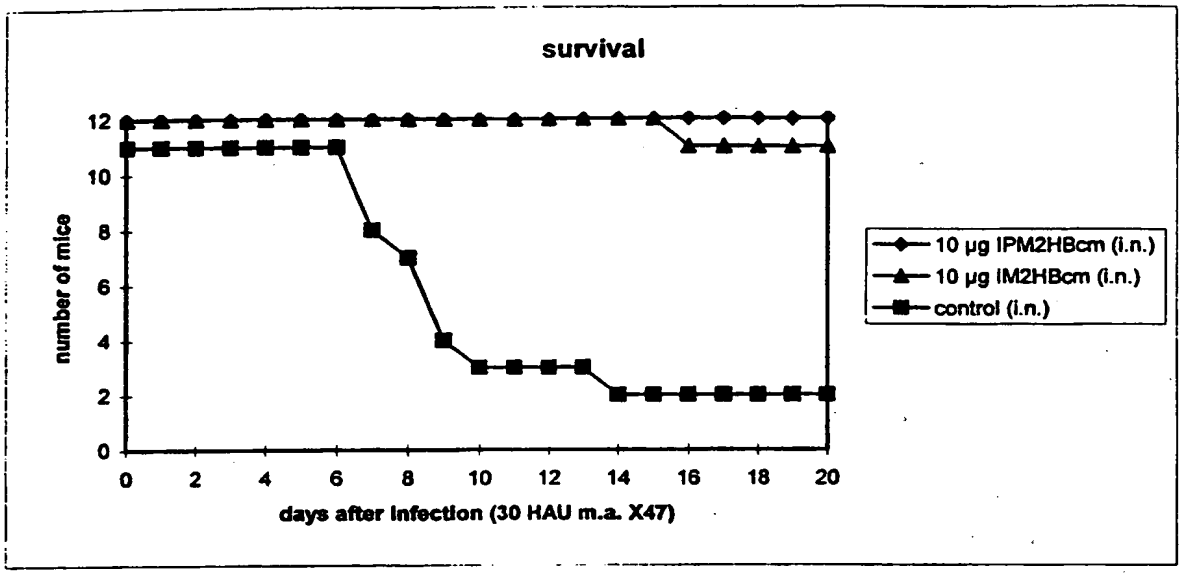

A
Rectal temperature
1.
T (°C)
39
37
35
33
31
29
27
25
0 2 4 6 8 10 12 14 16
days after infection
Weight
2.
m (g)
24
22
20
18
16
14
12
10
0 2 4 6 8 10 12 14 16
days after infection
B
Survival
number of mice
12
10
8
6
4
2
0
0 2 4 6 8 10 12 14 16
days after infection
50 µg IPM2HBcm
10 µg IPM2HBcm
5 µg IPM2HBcm
Control

fig.8D



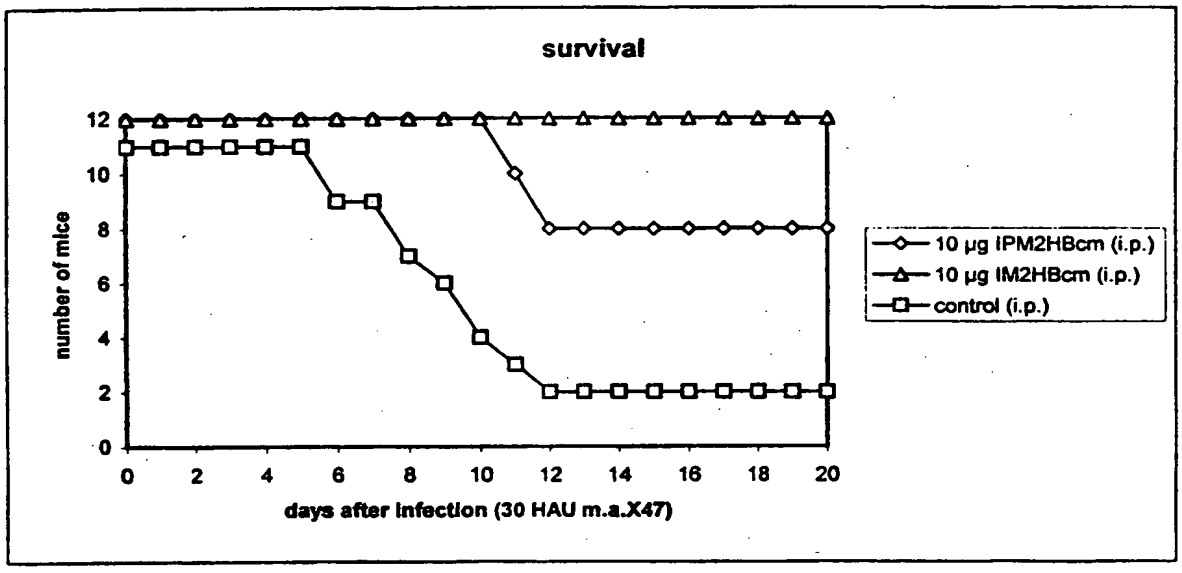


fig.8C

	surviving mice
10 µg IPM2HBcm (i.n.)	12/12
10 µg IM2HBcm (i.n.)	11/12
control (i.n.)	2/11
10 µg IPM2HBcm (i.p.)	8/12
10 µg IM2HBcm (i.p.)	12/12
control (i.p.)	2/12

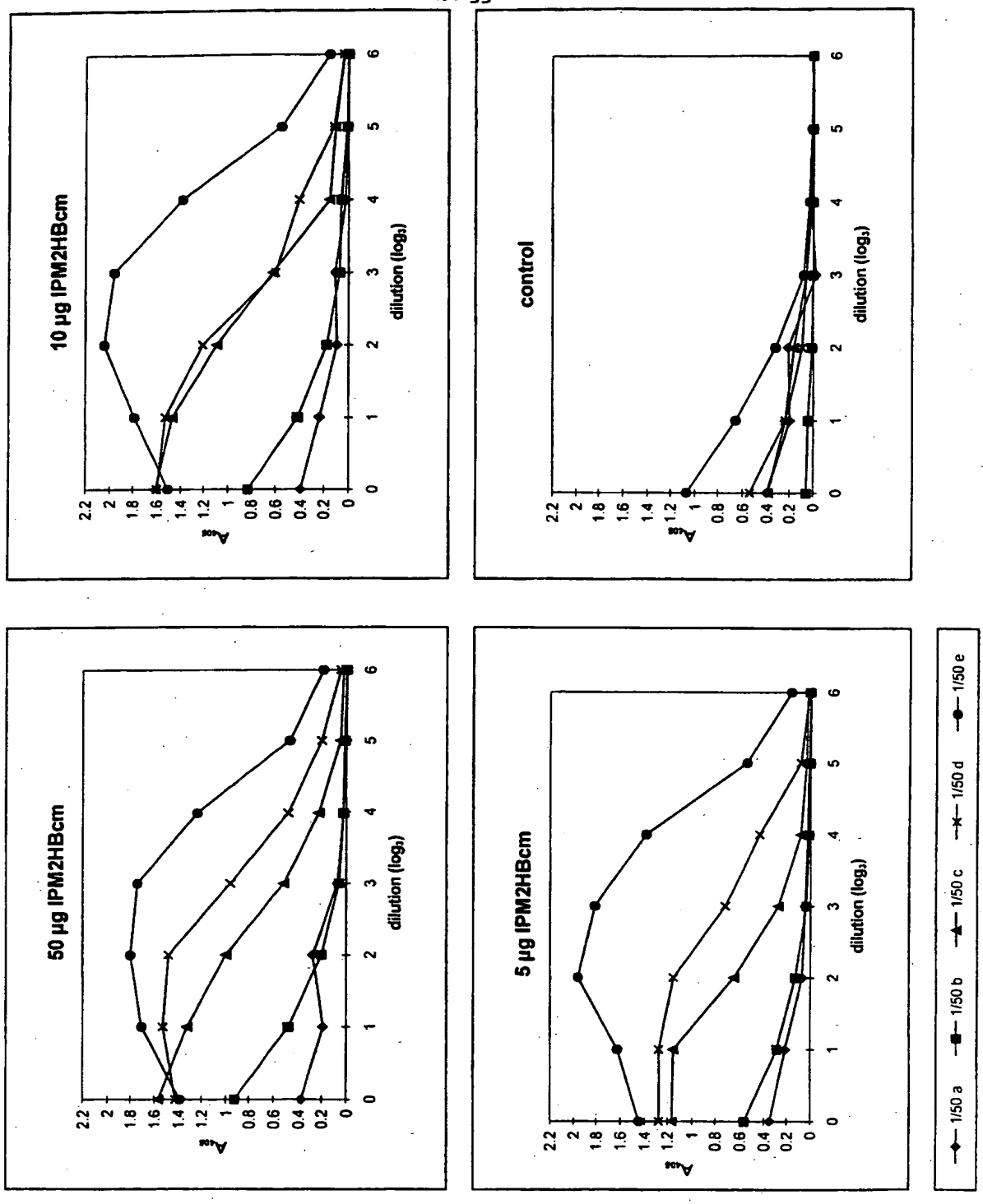

10 µg IPM2HBcm
control
50 µg IPM2HBcm
5 µg IPM2HBcm
dilution (log2)
A405
1/50 a
1/50 b
1/50 c
1/50 d
1/50 e

Figure 9

Figure 10

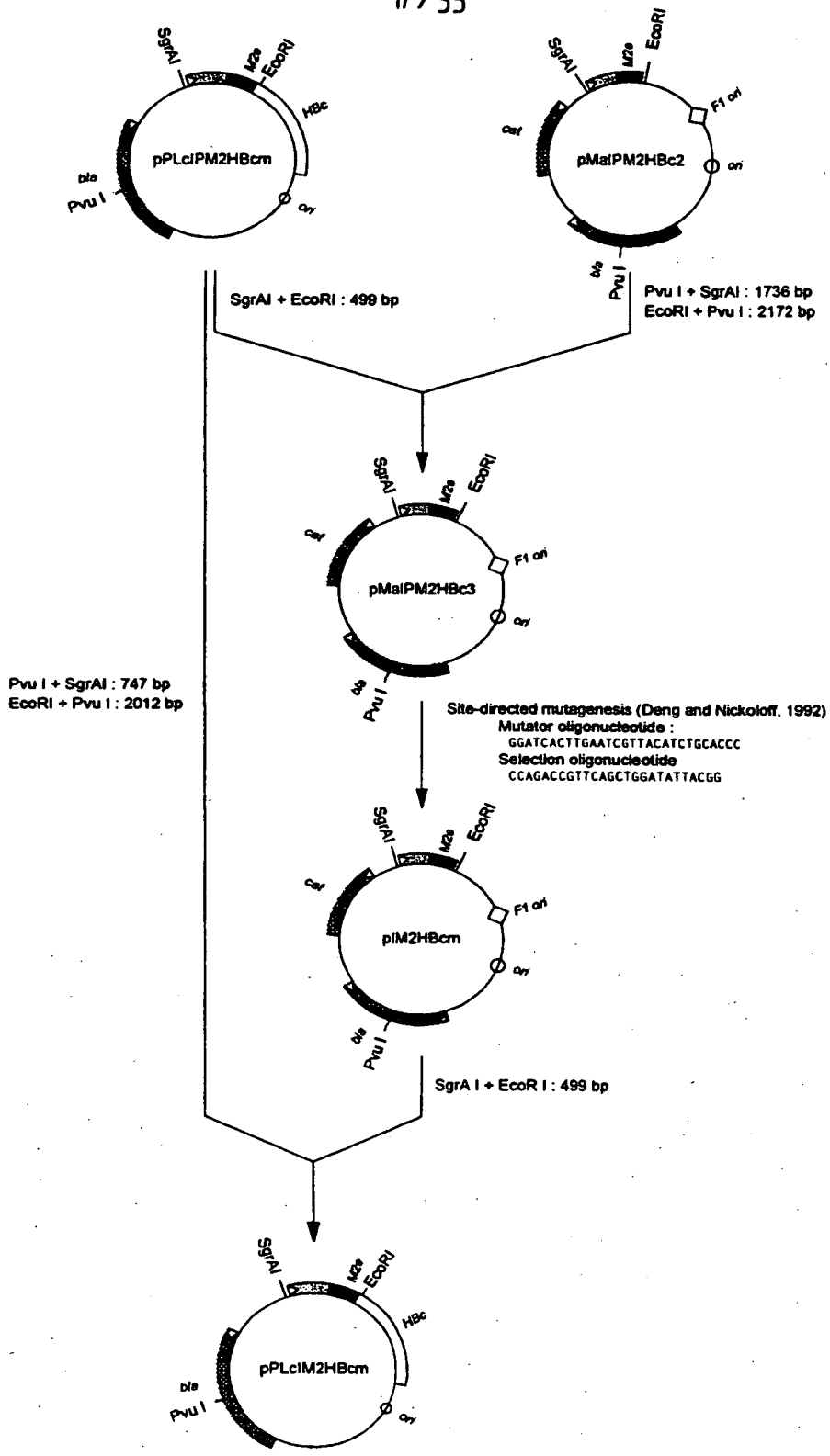

pPLclPM2HBcm
pMalPM2HBc2
SgrAI + EcoRI : 499 bp
Pvu I + SgrAI : 1736 bp
EcoRI + Pvu I : 2172 bp
pMalPM2HBc3
Pvu I + SgrAI : 747 bp
EcoRI + Pvu I : 2012 bp
Site-directed mutagenesis (Deng and Nickoloff, 1992)
Mutator oligonucleotide :
GGATCACTTGAATCGTTACATCTGCACCC
Selection oligonucleotide
CCAGACCGTTCAGCTGGATATTACGG
pIM2HBcm
SgrA I + EcoR I : 499 bp
pPLclM2HBcm

Figure 11

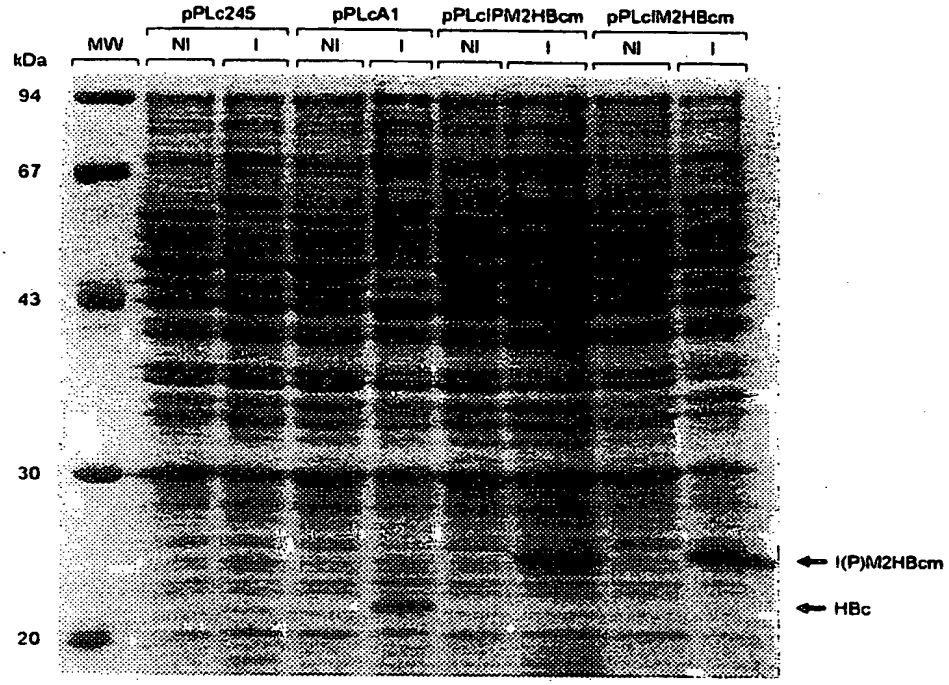
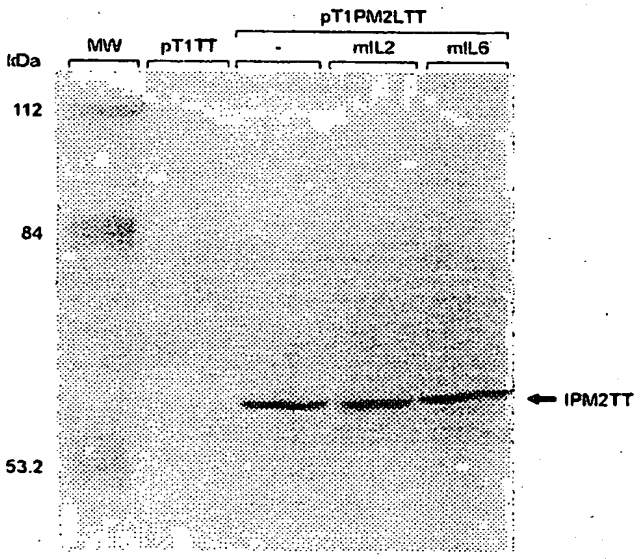


Figure 21



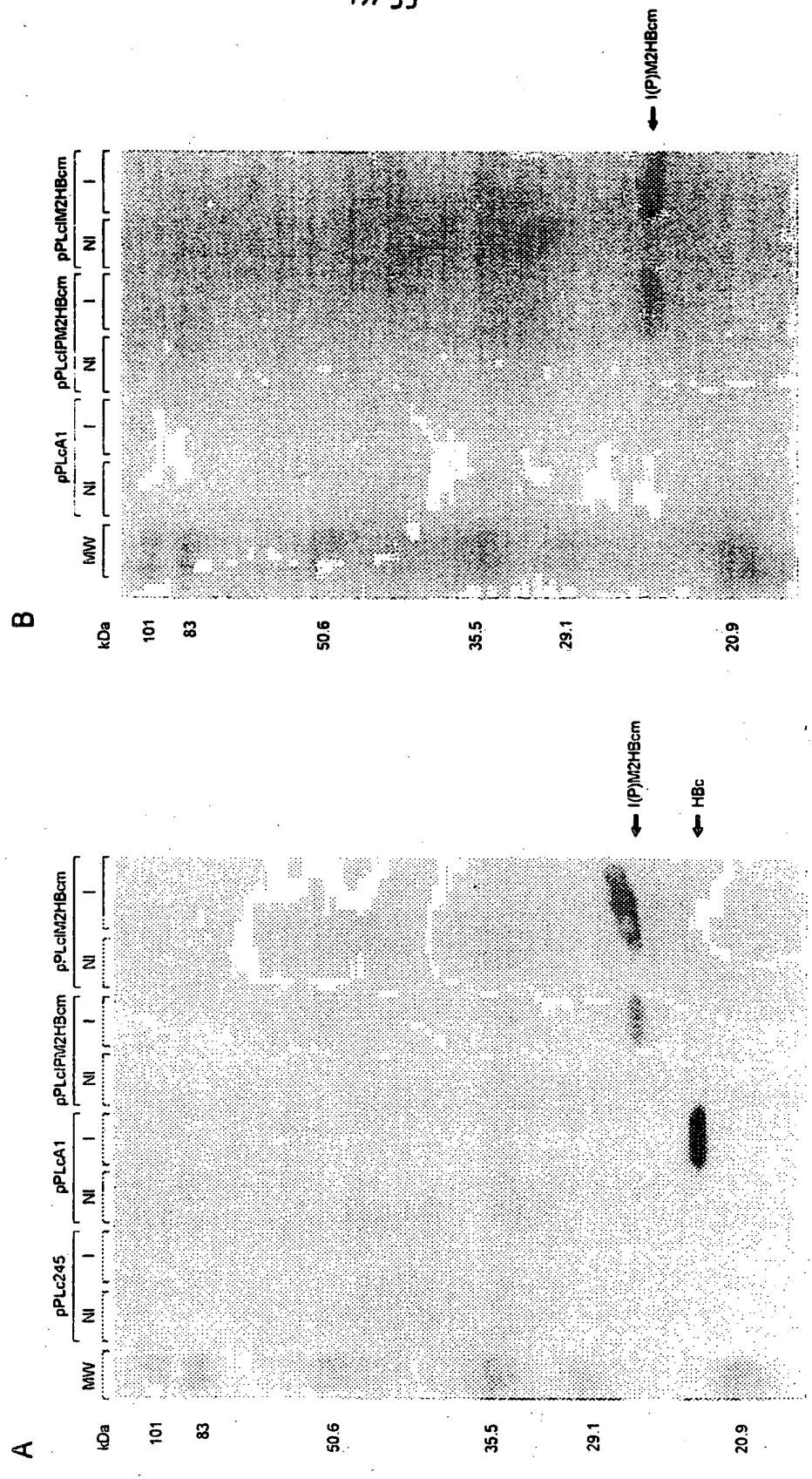

A
B
kDa
101
83
50.6
35.5
29.1
20.9
MW
pPLc245
pPLcA1
pPLcIPM2HBcm
pPLcIM2HBcm
NI
I
I(P)M2HBcm
HBc

Figure 12

Figure 13

Ersatzblatt (Regel 26)

HBcs (27-mer)

CATGGATATGGATCCTTATAAAGAATT
start

M2s (23-mer)

CATGTCTCTGCTGACCGAAGTTG
start

M2Ls (29-mer)

CATGTCTTTATTAACCGAAGTTGAAACCC
start

HBca (39-mer)

CGTGATCAACTAGTTCACTAACATTGAGATTCCCGAGAT
Bcl I Spe I stop

Figure 14

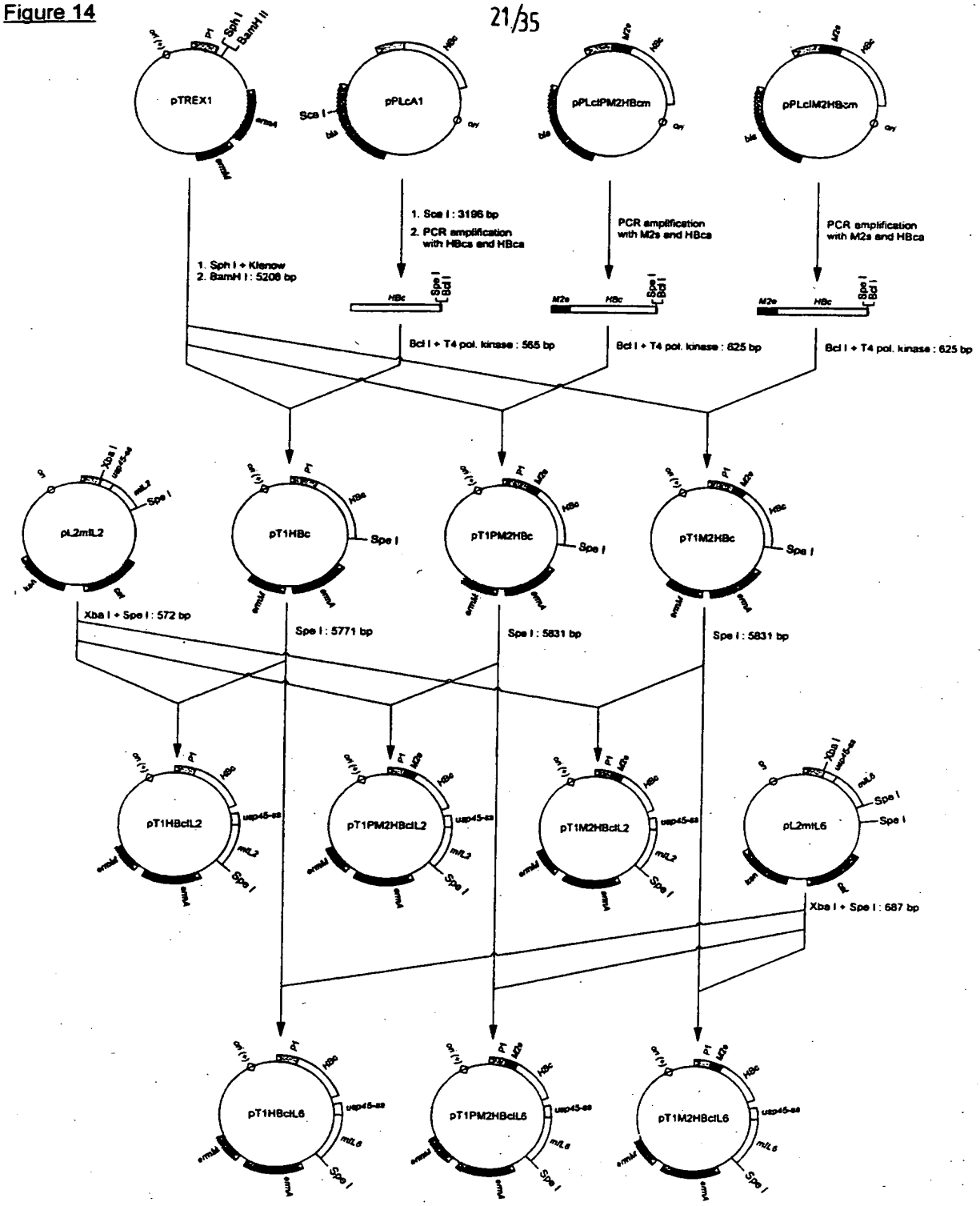

pTREX1
Sph I
BamH II
pPLcA1
Sca I
pPLclPM2HBcm
pPLclM2HBcm
1. Sca I : 3196 bp
2. PCR amplification with HBca and HBca
PCR amplification with M2s and HBca
PCR amplification with M2s and HBca
1. Sph I + Klenow
2. BamH I : 5208 bp
Spe I
Bcl I
Bcl I + T4 pol. kinase : 565 bp
Bcl I + T4 pol. kinase : 625 bp
Bcl I + T4 pol. kinase : 625 bp
pL2mIL2
pT1HBc
pT1PM2HBc
pT1M2HBc
Xba I + Spe I : 572 bp
Spe I : 5771 bp
Spe I : 5831 bp
Spe I : 5831 bp
pT1HBcIL2
pT1PM2HBcIL2
pT1M2HBcIL2
pL2mIL6
Xba I + Spe I : 687 bp
pT1HBcIL6
pT1PM2HBcIL6
pT1M2HBcIL6

Figure 15

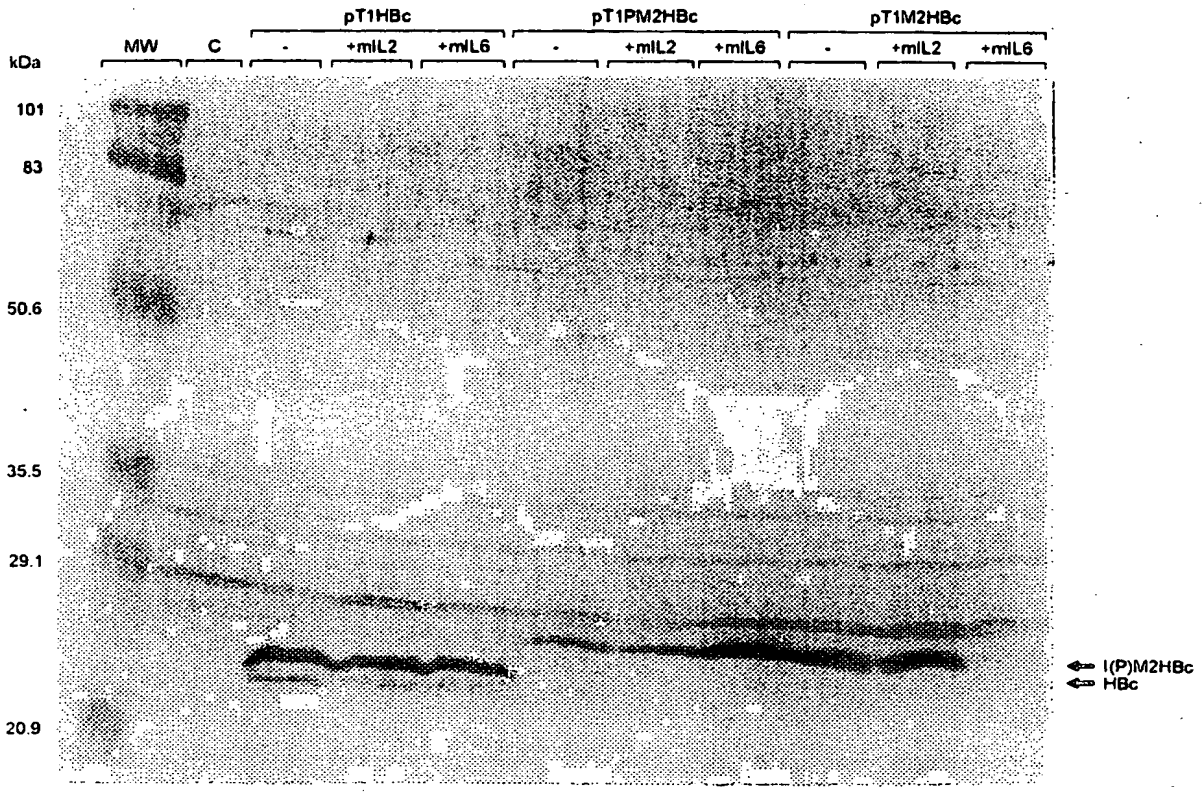


Figure 16

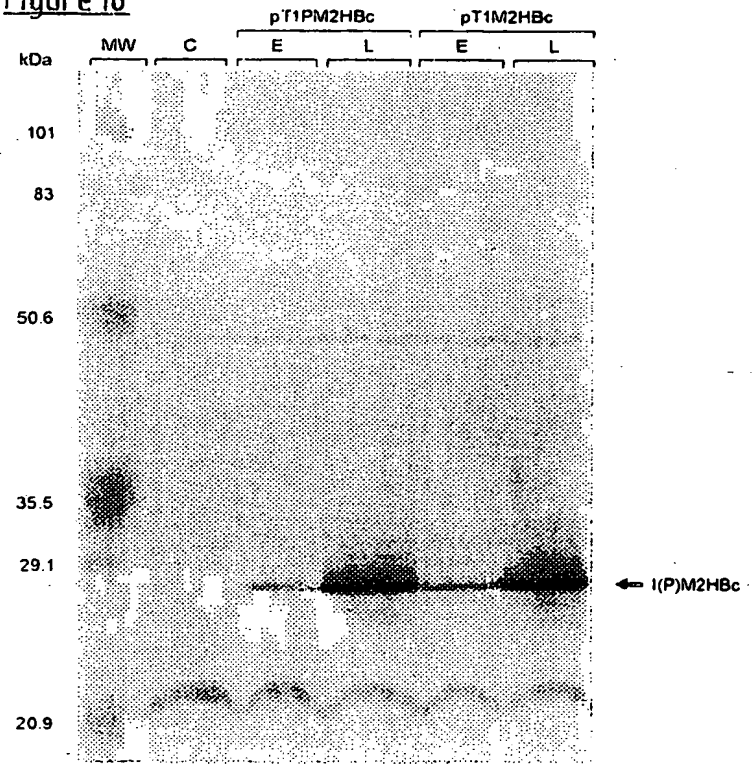


Figure 17

M2Ca (33-mer)

CGGGATCCCCACTTGAATCGTTACATCTGCACC

BamH I

M2LSs (30-mer)

TCTTTATTAACCGAAGTTGAAACCCCTATC

Ser

C3ds (35-mer)

CCGCGCCCACCCGACGAGATCTCGGATCTACCCCC

Bgl II

C3da (38-mer)

GCACTAGTTCAAGGATCCGATCCGAACTCTTCAGATCC

Spe I stop BamH I

Figure 18

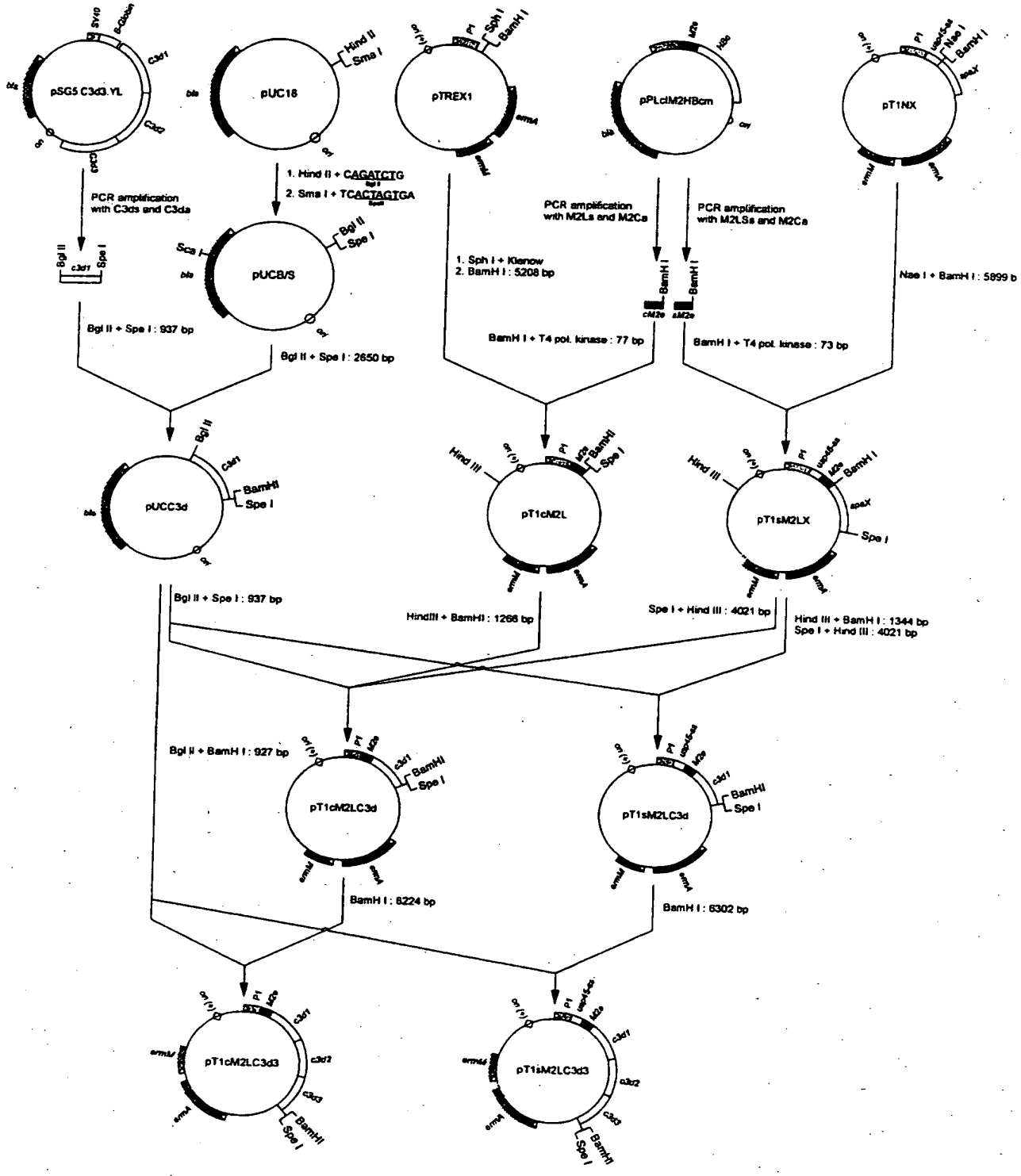

pSG5 C3d3.YL
pUC18
pTREX1
pPLcIM2HBcm
pT1NX
PCR amplification with C3ds and C3da
1. Hind II + CAGATCTG
2. Sma I + TCACTAGTGA
PCR amplification with M2Ls and M2Ca
PCR amplification with M2LSs and M2Ca
pUC8/S
1. Sph I + Klenow
2. BamH I : 5208 bp
Nae I + BamH I : 5899 b
Bgl II + Spe I : 937 bp
Bgl II + Spe I : 2650 bp
BamH I + T4 pol. kinase : 77 bp
BamH I + T4 pol. kinase : 73 bp
pUCC3d
pT1cM2L
pT1sM2LX
HindIII + BamHI : 1266 bp
Spe I + Hind III : 4021 bp
Hind III + BamH I : 1344 bp
Spe I + Hind III : 4021 bp
Bgl II + BamH I : 927 bp
pT1cM2LC3d
pT1sM2LC3d
BamH I : 8224 bp
BamH I : 6302 bp
pT1cM2LC3d3
pT1sM2LC3d3

Figure 19

TTFCs (35-mer)

CGGGATCCGACACCAATTCCATTTTCTTATTCTAA
BamH I

TTFCa (25-mer)

GGGGATCCACTAGTTTAATCATTTG
Bcl I Spe I stop

M2Ls (29-mer)

CATGTCTTTATTAACCGAAGTTGAAACCC
start

SUBSTITUTE SHEET (RULE 26)

Figure 20

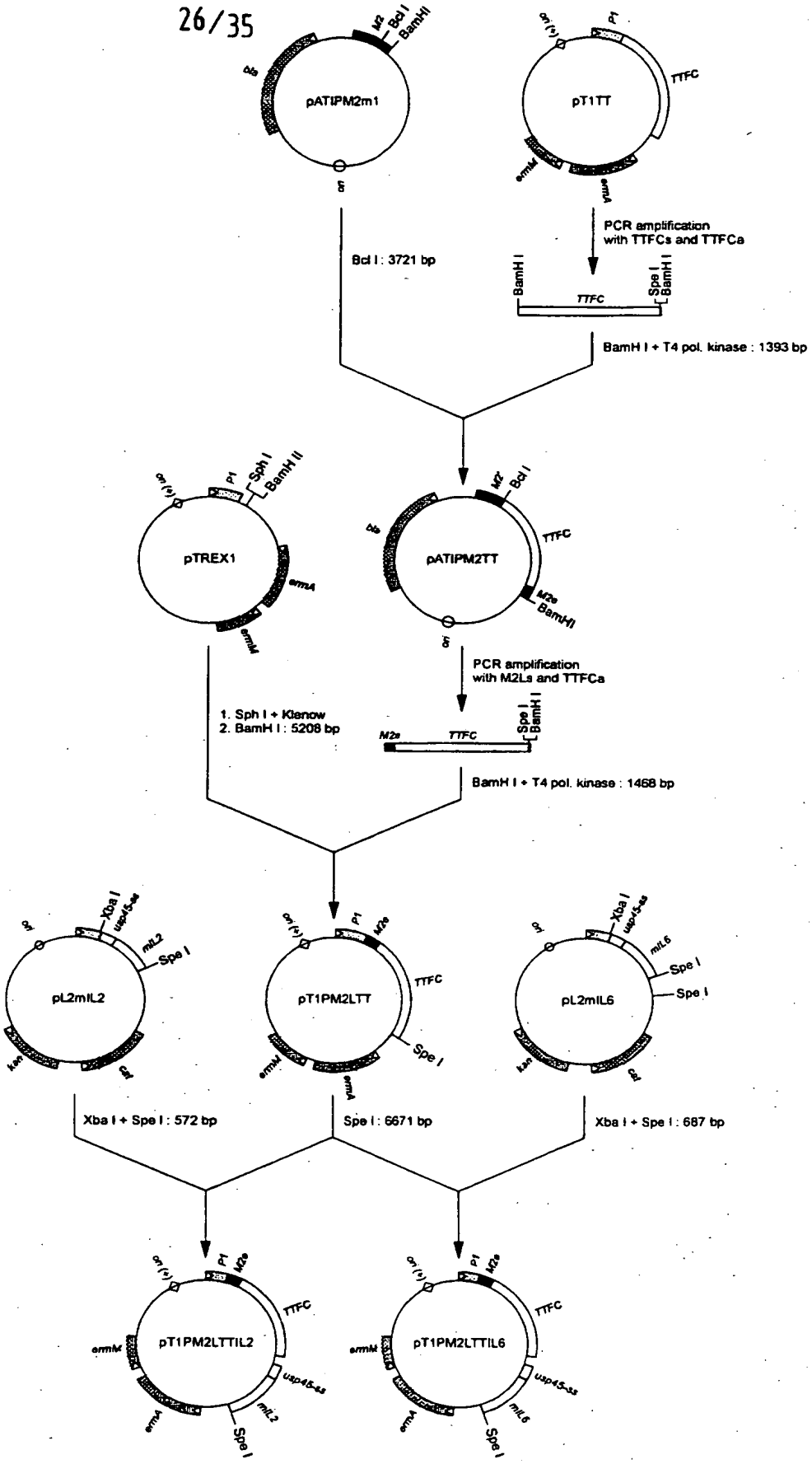

pATIPM2m1
M2
Bcl I
BamHI
δs
δ
pT1TT
ori (+)
P1
TTFC
ermM
ermA
PCR amplification with TTFCs and TTFCa
BamH I
TTFC
Spe I
BamH I
Bcl I : 3721 bp
BamH I + T4 pol. kinase : 1393 bp
pTREX1
ori (+)
P1
Sph I
BamH II
ermA
ermM
pATIPM2TT
M2
Bcl I
δs
TTFC
M2s
BamHI
δ
PCR amplification with M2Ls and TTFCa
M2s
TTFC
Spe I
BamH I
1. Sph I + Klenow
2. BamH I : 5208 bp
BamH I + T4 pol. kinase : 1468 bp
pL2mIL2
ori
Xba I
usp45-ss
mIL2
Spe I
kan
cat
pT1PM2LTT
ori (+)
P1
M2s
TTFC
Spe I
ermA
pL2mIL6
ori
Xba I
usp45-ss
mIL6
Spe I
Spe I
kan
cat
Xba I + Spe I : 572 bp
Spe I : 6671 bp
Xba I + Spe I : 687 bp
pT1PM2LTTIL2
ori (+)
P1
M2s
TTFC
ermM
usp45-ss
ermA
mIL2
Spe I
pT1PM2LTTIL6
ori (+)
P1
M2s
TTFC
ermM
usp45-ss
ermA
mIL6
Spe I

Figure 22

GP67s (25-mer)
GCTACTAGTAAATCAGTCACACCAA
SpeI

GP67a (33-mer)
CGAAGCTTGCCGGCAAAGGCAGAATGCGCCGCC
HinDIII NaeI

Figure 23

M2Ss (23-mer)
TCTCTGCTGACCGAAGTTGAAAC

UM2ECa (50-mer)
CGAAGCTTACTAGTTCACGGATCCCCACTTGAATCGTTGCATCTGCACCC
HindIII SpeI stop BamHI

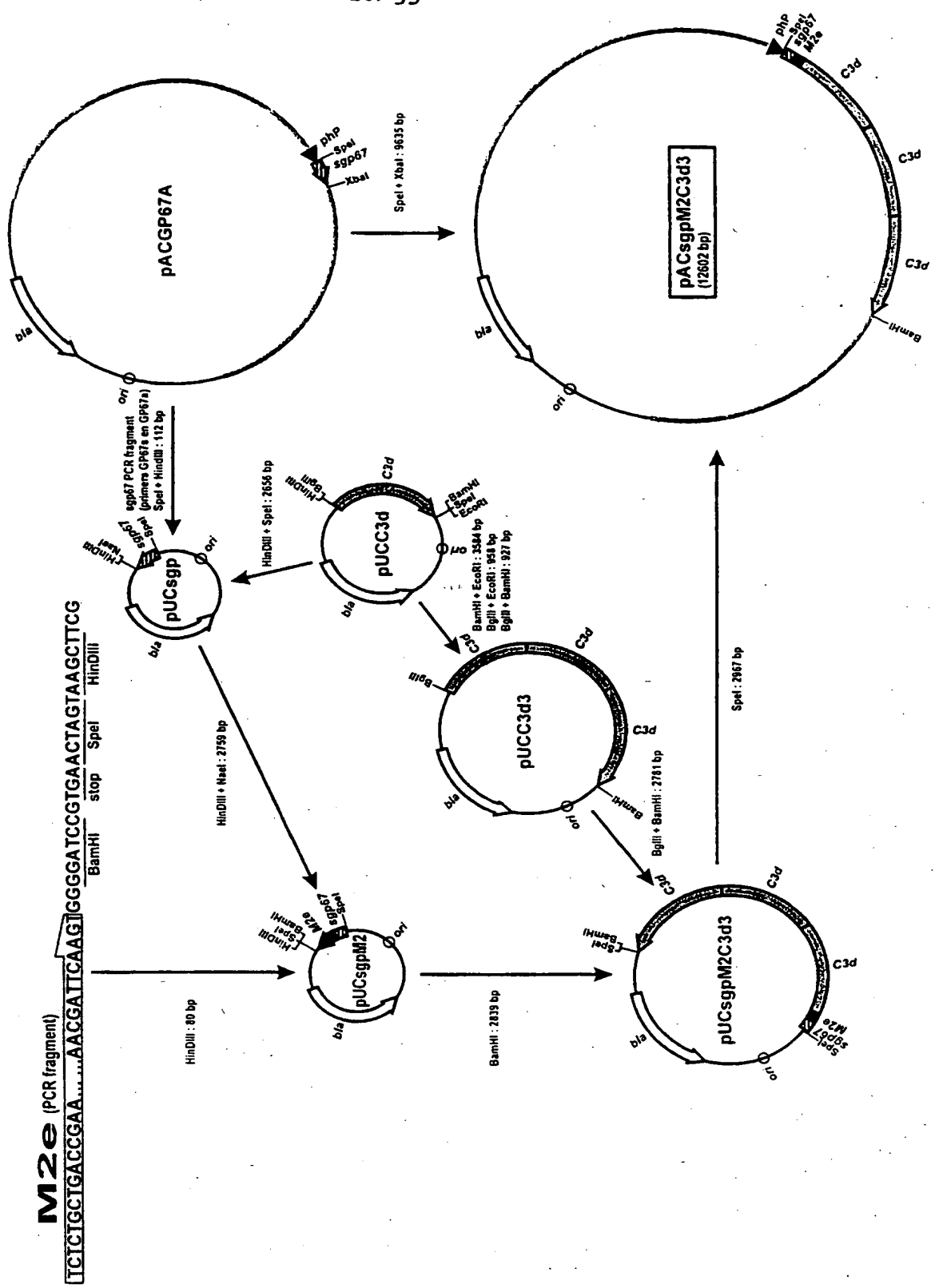

M2e (PCR fragment)
TCTCTGCTGACCGAA.....AACGATTCAAGTGGGGATCCGTGAACTAGTAAGCTTCG
BamHI stop SpeI HinDIII
pACGP67A
php
SpeI
sgp67
XbaI
bla
ori
Spel + Xbal : 9635 bp
pACsgpM2C3d3
(12602 bp)
C3d
BamHI
sgp67 PCR fragment
(primers GP67e en GP67a)
SpeI + HindIII : 112 bp
pUCsgp
HinDIII
NaeI
SpeI
sgp67
HinDIII + SpeI : 2656 bp
pUCC3d
BglII
BamHI
SpeI
EcoRI
BamHI + EcoRI : 3584 bp
BglII + EcoRI : 958 bp
BglII + BamHI : 927 bp
pUCC3d3
BglII
BamHI
BglII + BamHI : 2781 bp
HinDIII + NaeI : 2759 bp
SpeI : 2967 bp
HinDIII : 80 bp
pUCsgpM2
sgp67
M2e
BamHI
BamHI : 2839 bp
pUCsgpM2C3d3
SpeI
sgp67
M2e

Figure 24

Figure 25

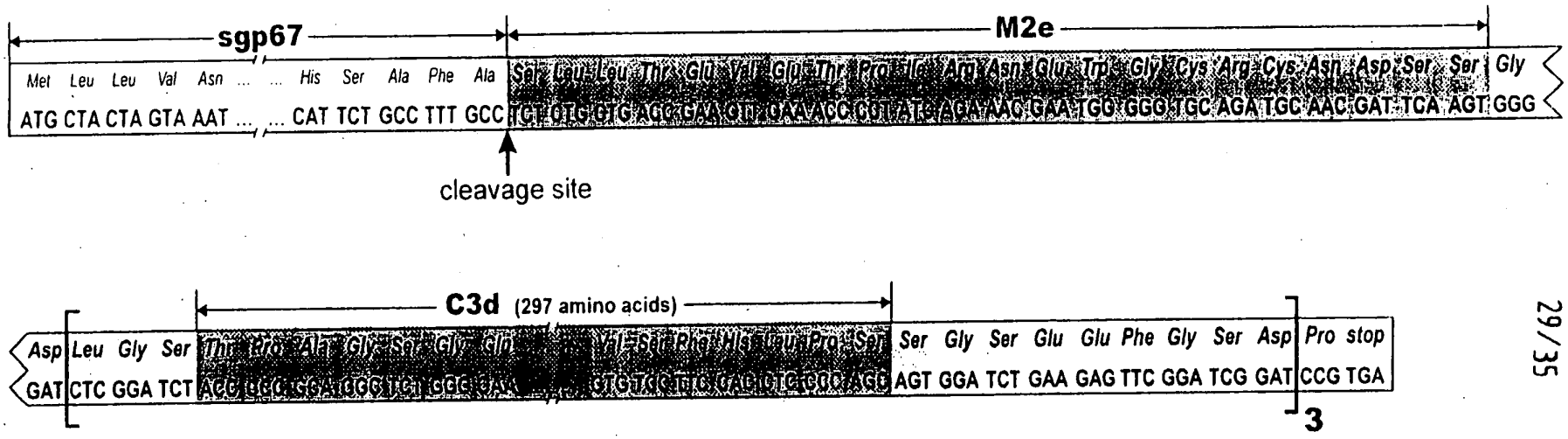

sgp67
M2e
Met Leu Leu Val Asn ... ... His Ser Ala Phe Ala Ser Leu Leu Thr Glu Val Glu Thr Pro Ile Arg Asn Glu Trp Gly Cys Arg Cys Asn Asp Ser Ser Gly
ATG CTA CTA GTA AAT ... ... CAT TCT GCC TTT GCC TCT CTG CTG ACC GAA GTT GAA ACC CCT ATA AGA AAC GAA TGG GGG TGC AGA TGC AAC GAT TCA AGT GGG
cleavage site
C3d (297 amino acids)
Asp Leu Gly Ser Thr Pro Ala Gly Ser Gly Gln Val Ser Phe His Leu Pro Ser Ser Gly Ser Glu Glu Phe Gly Ser Asp Pro stop
GAT CTC GGA TCT ACC CCG GCA GGA TCT GGC CAA GTG TCC TTC CAC CTG CCC AGC AGT GGA TCT GAA GAG TTC GGA TCG GAT CCG TGA
3

Figure 26

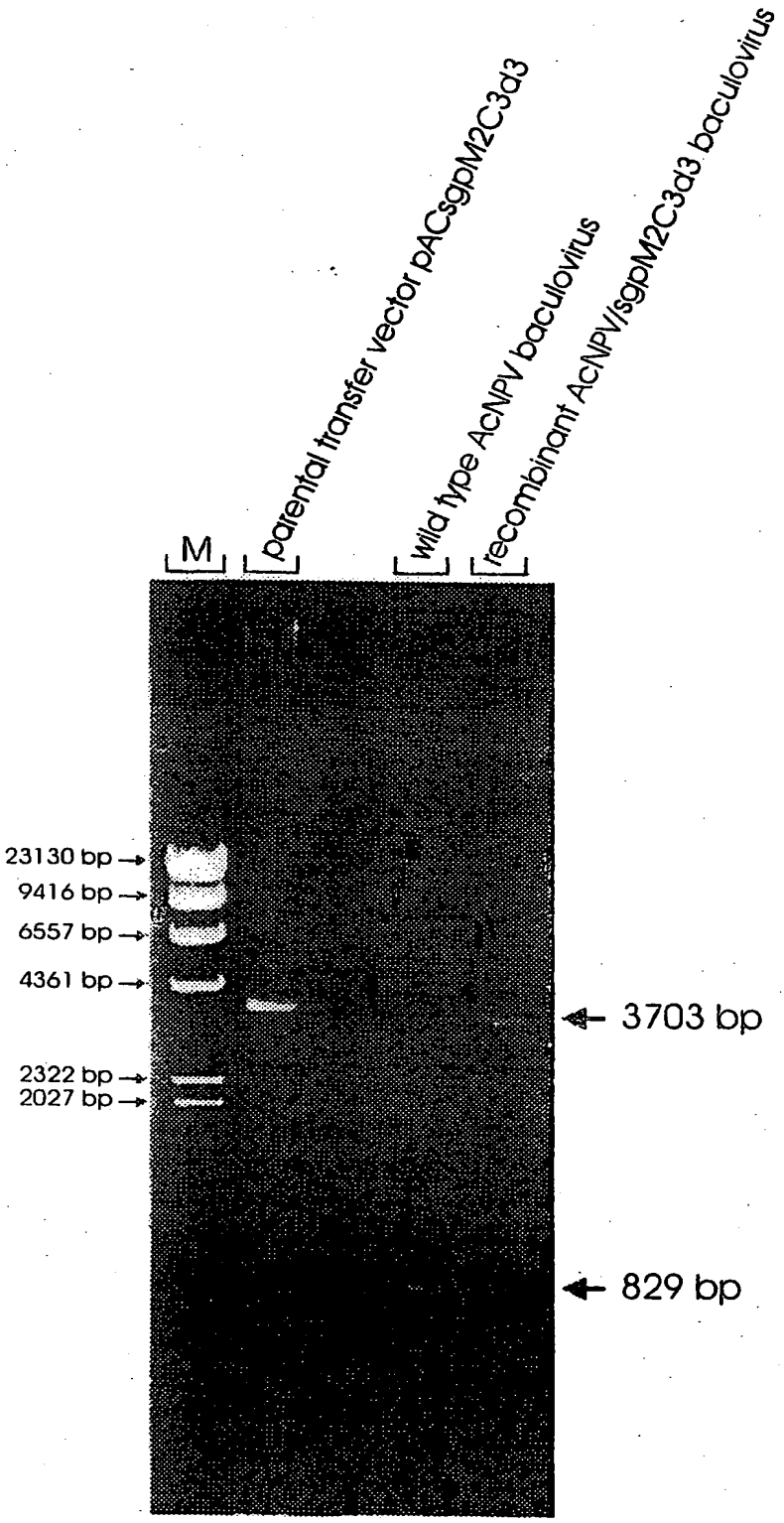

M
parental transfer vector pACsgpM2C3d3
wild type AcNPV baculovirus
recombinant AcNPV/sgpM2C3d3 baculovirus
23130 bp
9416 bp
6557 bp
4361 bp
2322 bp
2027 bp
3703 bp
829 bp

Figure 27

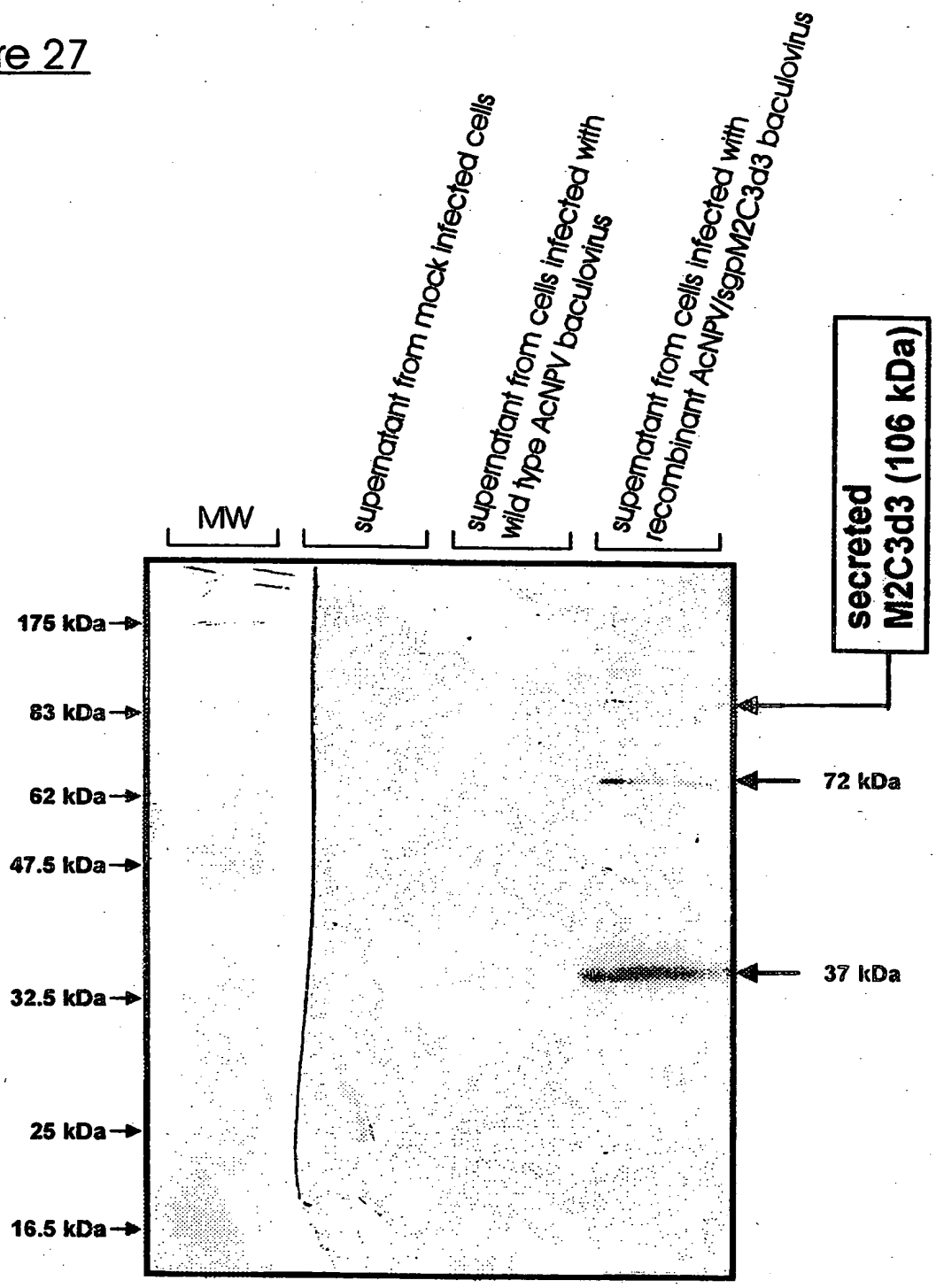

MW
supernatant from mock infected cells
supernatant from cells infected with wild type AcNPV baculovirus
supernatant from cells infected with recombinant AcNPV/sgpM2C3d3 baculovirus
secreted M2C3d3 (106 kDa)
175 kDa
83 kDa
62 kDa
47.5 kDa
32.5 kDa
25 kDa
16.5 kDa
72 kDa
37 kDa

Figure 28

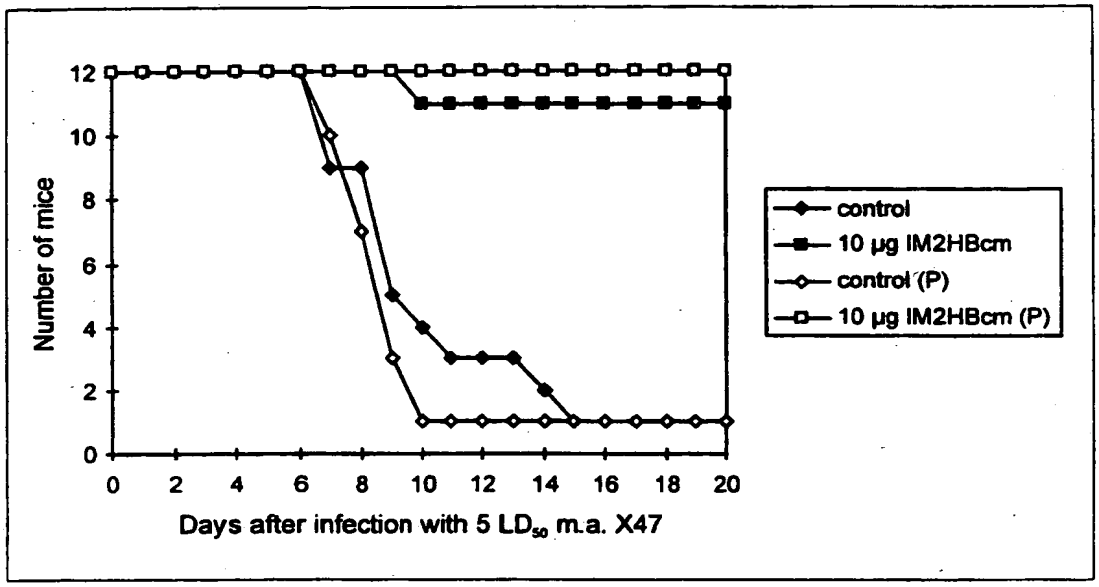

Number of mice
0
2
4
6
8
10
12
0
2
4
6
8
10
12
14
16
18
20
Days after infection with 5 LD50 m.a. X47
control
10 µg IM2HBcm
control (P)
10 µg IM2HBcm (P)

Figure 29

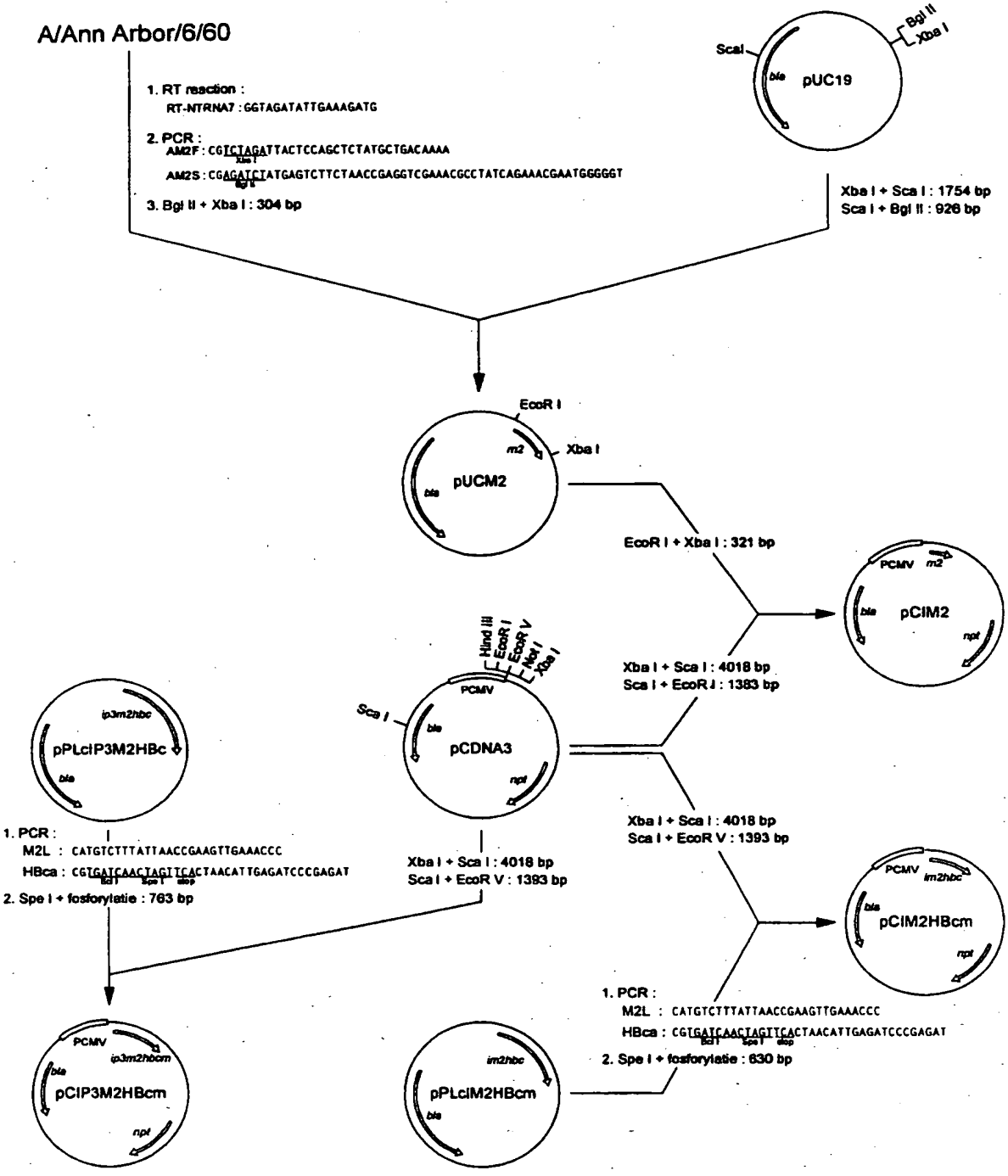

A/Ann Arbor/6/60
1. RT reaction :
RT-NTRNA7 : GGTAGATATTGAAAGATG
2. PCR :
AM2F : CGTCTAGATTACTCCAGCTCTATGCTGACAAAA
AM2S : CGAGATCTATGAGTCTTCTAACCGAGGTCGAAACGCCTATCAGAAACGAATGGGGGT
3. Bgl II + Xba I : 304 bp
ScaI
Bgl II
Xba I
pUC19
Xba I + Sca I : 1754 bp
Sca I + Bgl II : 926 bp
EcoR I
Xba I
pUCM2
EcoR I + Xba I : 321 bp
PCMV
m2
pCIM2
Hind III
EcoR I
EcoR V
Nar I
Xba I
Sca I
PCMV
pCDNA3
Xba I + Sca I : 4018 bp
Sca I + EcoR I : 1383 bp
Xba I + Sca I : 4018 bp
Sca I + EcoR V : 1393 bp
ip3m2hbc
pPLclP3M2HBc
1. PCR :
M2L : CATGTCTTTATTAACCGAAGTTGAAACCC
HBca : CGTGATCAACTAGTTCACTAACATTGAGATCCCGAGAT
2. Spe I + fosforylatie : 763 bp
Xba I + Sca I : 4018 bp
Sca I + EcoR V : 1393 bp
PCMV
im2hbc
pCIM2HBcm
1. PCR :
M2L : CATGTCTTTATTAACCGAAGTTGAAACCC
HBca : CGTGATCAACTAGTTCACTAACATTGAGATCCCGAGAT
2. Spe I + fosforylatie : 630 bp
PCMV
ip3m2hbcm
pCIP3M2HBcm
im2hbc
pPLclM2HBcm
bla
npt

Figure 30

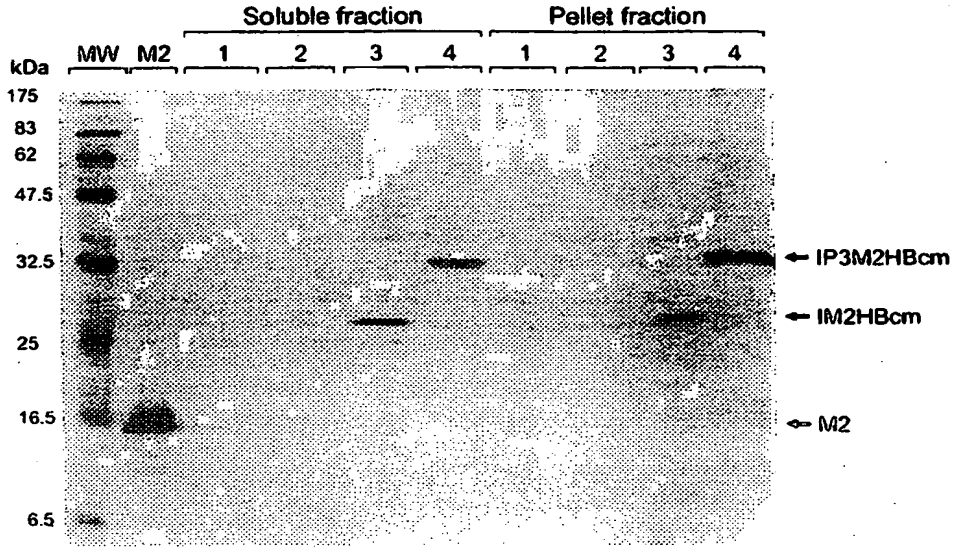
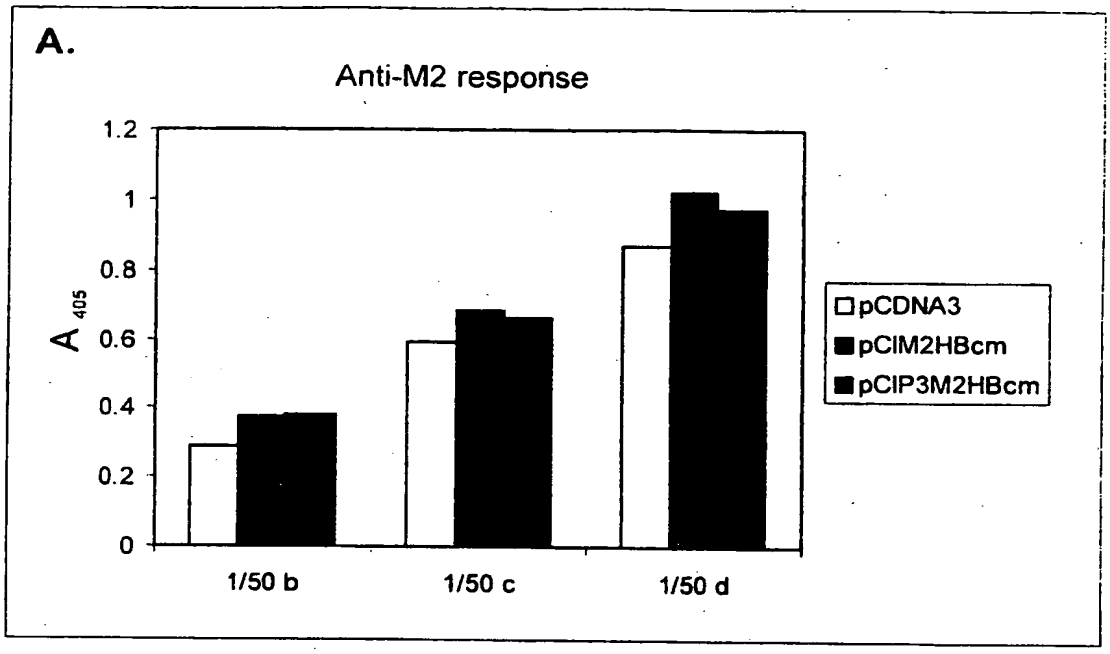
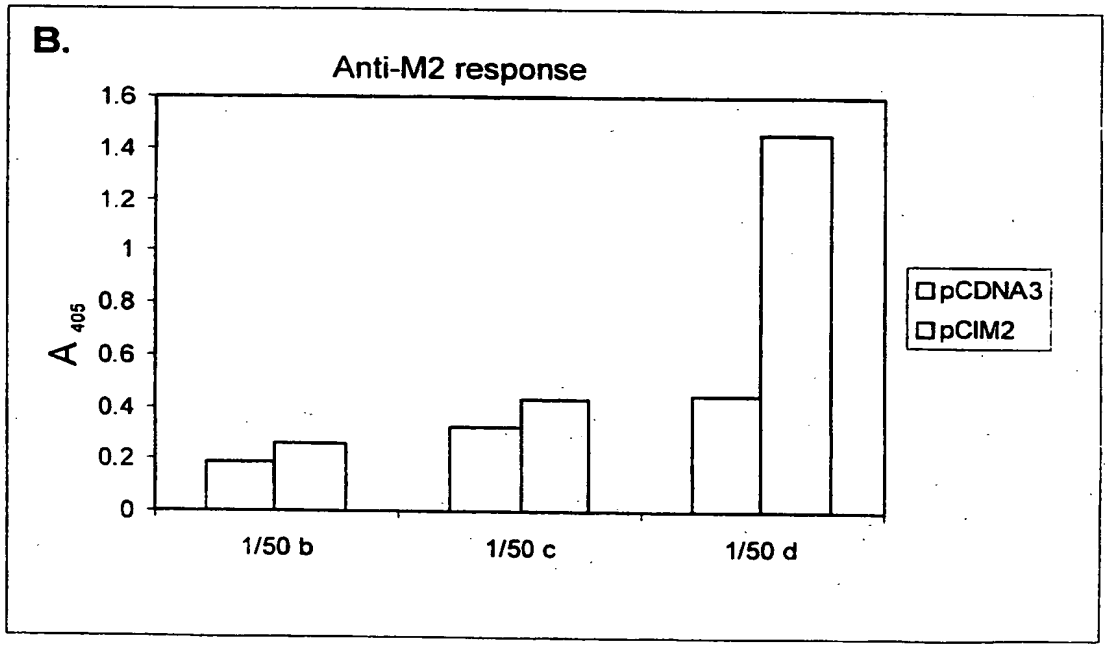

Soluble fraction
Pellet fraction
MW
M2
1
2
3
4
1
2
3
4
kDa
175
83
62
47.5
32.5
25
16.5
6.5
IP3M2HBcm
IM2HBcm
M2

Figure 31


A.
Anti-M2 response
1.2
1
0.8
0.6
0.4
0.2
0
A 405
1/50 b
1/50 c
1/50 d
pCDNA3
pCIM2HBcm
pCIP3M2HBcm


B.
Anti-M2 response
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A 405
1/50 b
1/50 c
1/50 d
pCDNA3
pCIM2